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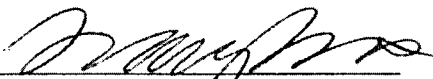
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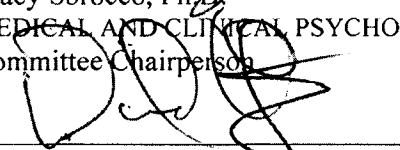
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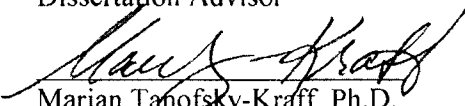
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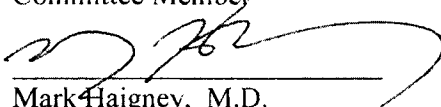
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Sari D. Holmes

Department of Medical and Clinical Psychology

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## **ABSTRACT**

Title of Thesis: B-Type Natriuretic Peptide Reactivity to Mental Stress  
and Exercise: Role of Obesity and Hemodynamics

Author: Sari D. Holmes, Doctor of Philosophy, 2009

Thesis directed by: David S. Krantz, Ph.D.  
Professor and Chair  
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The prevalence of heart failure in the United States is estimated at 5.2 million individuals, and although survival has increased, the incidence of heart failure remains steady. Obesity is an important risk factor for the development of heart failure and other cardiovascular diseases and evidence suggests that obese individuals respond to stressors differently from lean individuals. Psychosocial factors, including stress, are related to decompensation in heart failure and to increased incidence of cardiovascular events. However, it remains unclear how the variables of obesity and stress interact to affect cardiovascular disease risk and the mechanisms involved.

Mental stressors (cognitive/emotional and psychomotor) were presented to 33 participants to assess reactivity (acute changes) in heart rate, blood pressure, cardiac output, total peripheral resistance, and B-type natriuretic peptide (BNP). Reactivity to these stressors was also compared across body mass index (BMI) derived weight groups: obese ( $\text{BMI} \geq 30 \text{ kg/m}^2$ ), overweight ( $25 \leq \text{BMI} < 30 \text{ kg/m}^2$ ), and normal weight ( $18.5 \leq \text{BMI} < 25 \text{ kg/m}^2$ ).

The cognitive/emotional tasks significantly increased cardiac output, whereas the mirror trace task showed a significant total peripheral resistance increase. BNP increased significantly with mental stress, but BNP reactivity did not differ between the two types of mental stress. The increase in BNP with exercise was significant and markedly larger than with the mental stress tasks. There were no significant associations between stress reactivity and the three weight groups. However, in females, waist-to-hip ratio (WHR) was correlated with greater TPR reactivity, BNP at baseline, and BNP reactivity.

In summary, mental stress tasks significantly increased BNP, although to a lesser extent than exercise. In females, WHR was found to be a better indicator of hemodynamic and BNP reactivity than BMI or weight groups. Mental stress reactivity of BNP and WHR may therefore be useful in the prediction of cardiovascular disease.

**B-Type Natriuretic Peptide Reactivity to Mental Stress and Exercise:  
Role of Obesity and Hemodynamics**

by

Sari D. Holmes

Dissertation submitted to the faculty of the  
Department of Medical and Clinical Psychology  
Graduate Program of the Uniformed Services University  
of the Health Sciences in partial fulfillment  
of the requirements for the degree of  
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## Dedication

*The work presented here is dedicated to my husband, Andrew DeVoy Holmes. His unending support and love has been instrumental in navigating the often difficult and stressful moments of my graduate studies. Without his support I would not be the person or scientist I am today. Thank you! I love you to the moon and back.*

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## **Background**

The prevalence of heart failure in the United States is estimated at 5.2 million individuals (4). There are approximately 570,000 new cases of heart failure each year (4), and although survival has increased (5), the incidence of heart failure remains steady. In addition to hypertension and diabetes, obesity has been determined as an important risk factor for the development of heart failure (6). With 31% of Americans classified as obese, the study of obesity as a cardiovascular risk factor is important, particularly because cardiovascular responses to stressors and disease indicators function differently for obese individuals as compared to non-obese individuals (7, 8).

Evidence suggests that psychosocial factors, including stress and depression, are related to decompensation in heart failure (9, 10) and to increased incidence of cardiovascular events (11, 12). However, it remains unclear how the variables of obesity, stress, and depression interact to affect cardiovascular disease risk and the mechanisms involved. The present study aims to combine information from diverse research domains in order to evaluate traditional cardiovascular risk markers (i.e., blood pressure and cardiac output) in a broader context as well as investigate novel pathways and markers involving hemodynamic responses to stress. Therefore, this study will compare cardiovascular responses to two types of mental stress between obese and non-obese individuals while assessing the moderating role of depressive symptoms and subtypes.

The following sections will provide an overview of heart failure as well as a review of the literature in the areas of stress, obesity, and depression as they relate to cardiovascular disease risk and hemodynamic reactivity. This review will present a rationale for the research model and hypotheses around which the current study is based.

## **Heart failure**

### Definition, Etiology, and Pathophysiology

Heart failure is the final manifestation of many forms of cardiovascular disease. Heart failure is a clinical symptomatic syndrome resulting from “the inability of the heart to pump blood forward at a sufficient rate to meet the metabolic demands of the body (‘forward failure’), or the ability to do so only if the cardiac filling pressures are abnormally high (‘backward failure’), or both” (13). Clinically, heart failure is manifested by symptoms such as fatigue, dyspnea (shortness of breath), orthopnea (dyspnea when lying flat), paroxysmal nocturnal dyspnea (breathing difficulties after laying flat for extended time period), peripheral edema (accumulation of fluid in lower limbs), and right upper quadrant discomfort (due to enlargement of the liver) (13). For classification purposes, the New York Heart Association (NYHA) has graded the symptoms of heart failure into a system shown in Table 1 (1). When left ventricular function is compromised as in heart failure, the ejection fraction is reduced. Ejection fraction (EF) is the

percentage of blood in the ventricle at the end of diastole that is ejected from the ventricle during each systolic contraction.

Etiology. The etiology of heart failure can follow several possible pathways, resulting in either systolic or diastolic dysfunction. Systolic dysfunction describes a diminished capacity of an affected ventricle to eject blood whereas diastolic dysfunction refers to an abnormality in the filling or relaxation of the heart during diastole (13). Mechanisms involved in systolic dysfunction can be impaired contractility and pressure overload (increased afterload). Impaired contractility, due to the destruction or abnormal function of heart muscle cells or the development of excess fibrous tissue, can occur following a cardiac event (i.e., myocardial infarction or ischemia) or chronic volume overload. Increased afterload, the ventricular pressure needed to eject blood in systole, can result from uncontrolled hypertension and/or reduced blood flow due to incomplete aortic valve opening.

In contrast, the two main etiologies for diastolic dysfunction are impaired ventricular relaxation and obstruction of left ventricular filling. Impaired ventricular relaxation is mainly due to increased ventricular wall stiffness caused by: (1) left ventricular hypertrophy - thickening of the heart muscle in the left ventricle in response to pressure or volume overload; (2) cardiomyopathies - decline in heart muscle functionality; and (3) myocardial ischemia - inadequate blood flow to the heart causing an imbalance in oxygen supply and demand in the heart.

Obstruction of left ventricular filling occurs because of conditions such as mitral

stenosis (narrowing of the mitral valve opening) and tamponade (fluid fills the pericardial space), which prevent adequate blood flow in and out of the heart.

Secondary Changes in Heart Failure. When cardiac output is reduced, the body attempts to compensate with several mechanisms. An overview of the heart failure process is shown in Figure 1 (2). There is an initial event or disease state that results in decreased stroke volume and cardiac output. This initial impairment is followed by secondary changes to compensate for the reduced pumping ability. Examples of these secondary changes include: retaining salt and water to increase the amount of blood in the bloodstream, increasing heart rate, and increasing the size of the heart. As illustrated in Figure 1 (2), these secondary changes are regulated by activation of the renin-angiotension-aldosterone system (RAAS) and the sympathetic nervous system, as well as increased production of antidiuretic hormone (ADH). Further, remodeling of the heart, stretching of the heart muscle in response to excessive wall stress, results in increased heart size or ventricular hypertrophy. In addition, chronic inflammation is present in heart failure, evidenced by high levels of tumor necrosis factor alpha (TNF- $\alpha$ ) and other pro-inflammatory cytokines (14). This inflammation process contributes to remodeling of the heart, which results in the progression of symptoms and disease in heart failure (15). It is important to remember that the processes enlisted as compensatory mechanisms to promote increases in cardiac output and heart function in the short-term become detrimental when chronically activated and can lead to worsening heart failure.

## Epidemiology of Heart Failure

Research demonstrates that heart failure is a terminal condition with a survival rate only somewhat better than cancer (16). At five years after a heart failure diagnosis, only 50% of patients are alive, while patients with NYHA Class III or Class IV symptoms have a 1-year survival rate of 40% (13). Heart failure is associated with an annual mortality rate of 10%, even with the best therapy (17).

Unlike many of the other cardiac diseases, the incidence of heart failure is increasing due to the growing older population and the success of interventions on survival after cardiac events (13). Current reports show that there are approximately 570,000 new cases of heart failure each year (4). Barker and colleagues (18) found that between the study periods of 1970-1974 and 1990-1994, the age-adjusted incidence of heart failure increased 14% among persons 65 years or older. In contrast, a community-based cohort study found that the incidence of HF showed no change across two decades (1979-2000), but survival after onset did increase over time, less so among women and elderly persons (5).

The overall prevalence of heart failure in males aged 20+ (2.6 million, 2.8%) is slightly higher than in females (2.6 million, 2.2%) (4). Although the prevalence of heart failure is relatively similar across ethnic groups, there are gender differences among ethnic groups. While the heart failure prevalence in white males (2.8%) and Mexican American males (2.1%) is slightly higher than in white females (2.1%) and Mexican American females (1.9%) respectively, the

heart failure prevalence in black females (3.3%) is higher than in black males (2.7%) (4).

#### LV Dysfunction and Relevance of B-Type Natriuretic Peptide

Reduction in left ventricular (LV) function is the most common cause of symptoms and hospitalizations in heart failure patients with coronary artery disease (CAD). LV dysfunction is typically defined as an EF < 30%, which is seen in 2/3 of patients with HF (19). Myocardial ischemia, the imbalance between myocardial blood/oxygen supply and demand, can result in LV dysfunction and impaired cardiac pump function (20). LV remodeling and increased wall stress (or tension), due to repeated cardiac insults and/or compensatory measures, can lead to pump dysfunction and failure, as well as increased risk of arrhythmias (irregular electrical activity of the heart) and sudden death (21). Therefore, biomarkers of overload and wall stress are becoming increasingly important predictors of heart failure morbidity/mortality outcomes (22, 23). One such biomarker that has shown promise in the diagnosis and treatment of heart failure is B-type natriuretic peptide (BNP).

BNP is one of several peptide hormones (A-type, B-type, and C-type) secreted by the heart in response to volume and pressure overload and myocardial stretch (24). BNP is released from ventricular myocytes in response to myocyte stretching whereas ANP is released from atrial myocytes in response to high blood pressure. The natriuretic peptides are hormones with strong diuretic and natriuretic actions, the ability to relax vascular smooth muscle, and are

natural antagonists for the sympathetic nervous system and the RAAS (23). ANP and BNP decrease systemic vascular resistance and increase natriuresis (excretion of excess sodium in the urine) in order to decrease cardiac output and blood volume (25). The main physiological purpose of the natriuretic peptides is to maintain homeostasis and protect the cardiovascular system from volume overload (23). In effect, BNP (along with the other natriuretic peptides) counteracts the compensatory mechanisms described earlier that can worsen cardiac function when chronically activated and is therefore an important biomarker for use in heart failure diagnosis and treatment.

Clinically, BNP is important in diagnosis/differential diagnosis in individuals with suspected HF, with a decision cut-point of 100 pg/ml now accepted as positive for HF (23). Increases in BNP levels in the heart failure patient can also be used to track heart failure disease severity. For example, BNP consistently correlates with NYHA function class across a number of studies (26-28). Epidemiologic data from the Framingham study also showed that plasma BNP levels predicted death and cardiovascular events after adjusting for other risk factors, such that each one standard deviation increment in log BNP level was associated with a 27% increase in risk of death, a 28% increase in risk of first cardiovascular events, and a 77% increase in risk of HF (29). In addition, a doubling of BNP over baseline has been suggested as a clinically relevant marker of HF decompensation when taken together with information from the history and physical examination (23).

In sum, BNP appears to be a useful biomarker indicating decompensation in cardiac patients and may have implications/uses to address risk in healthy individuals.

## **Psychosocial Stress**

### Definition

The importance of stress on health and disease risk has been a topic of research interest for decades. Researchers have investigated the role of stress in many areas of health, including cancer (30, 31), cardiovascular disease (11), immune function (32), sleep (33, 34), and mental illness (35). In particular, coronary disease research has shown that stress is associated with every level of the disease process from its impact on risk factors to triggering of acute cardiac events. Stress is significantly associated with an increase in unhealthy behaviors such as smoking, alcohol consumption, poor diet, and reduced physical activity, which are all known risk factors for CAD (36-40). Stress is related to an increased inflammatory response, platelet aggregation, and endothelial dysfunction (41-43), which contribute to the development of coronary artery atherosclerosis (34) and coronary artery disease (25-33). In addition, acute events in CAD (i.e., myocardial ischemia and infarction, malignant arrhythmia, and sudden cardiac death) have been shown to be triggered acutely by stress (44-46).

The first step in exploring the role of stress in CAD is to fully understand “What is stress?” Scientists have conceptualized stress differently depending on the theoretical perspective they choose as a guide. The “environmental perspective” concerns how stressful life events are important in connecting the concepts of stress and disease, with particular focus on characteristics of the stressors. After decades of research aimed at describing the relationship between stressful life events and disease, the focus in recent years has turned to explaining the mechanisms involved in the association of disease and life events. Mechanisms to explain why individuals with similar life events can experience different outcomes have included reduced self-esteem, poor coping strategies, low social support, and an increased number of stressors (47).

Although research into the life events and illness relationship is valuable, Rabkin & Struening (48) found that no more than 9% of the variance in health outcomes is explained by life events. It is important to note that most individuals who experience stressors do not develop illness. Stressful life events are usually temporary, whereas other more traditional risk factors for disease can be longer-lasting and appear to be more influential for developing disease (i.e., smoking, alcohol consumption, a high-fat, low-fiber diet, and risky lifestyle) (49).

The “psychological stress perspective” describes how psychological variables associated with the person are responsible for the creation of a stress response to a particular environmental stressor. Researchers operating within this model are concerned with individual differences in appraisal, coping, and other cognitive variables as these concepts relate to the stressor-response

relationship. Ultimately, stress occurs when stimuli, events, and responsibilities exceed one's *perceived* coping abilities.

The next question is, "How is stress measured?" Just as the definition of stress has several conceptualizations, the measurement of stress can be carried out with a variety of different methods, including self-report, physiologic measurements, and behavioral/performance measures. Clinicians and researchers can tailor the stress measurement method to the definition of stress that is utilized and the question they are interested in investigating.

### Stress and Cardiovascular Disease

The last step in understanding stress is to answer the following question: "How does stress relate to and affect CAD?" There is an abundance of evidence in the literature supporting the notion that stress greatly impacts CAD (50-59). It has already been noted that stress may present in various forms, but the evidence linking stress and CAD crosses all definitions. Chronic stressors, in the form of job stress, marital stress, and the stress associated with poverty, have been associated with increased cardiovascular risk and incidence of CAD (50-52). In particular, low decision latitude in an individual's occupation was found to be associated with increased CHD morbidity and mortality (50). In addition, the primate literature shows that chronic stress, due to social disruption, is related to greater development of atherosclerosis (53).

Perhaps the most studied area in the stress literature is the cardiovascular effect of acute stress. Investigators have found that acute cardiovascular events,

such as myocardial ischemia and infarction, can be triggered by physical and mental stressors (45). In addition, several studies have shown that natural disasters (54, 55) and traumatic events (56, 57) are associated with increases in cardiac events, including myocardial infarction, ventricular arrhythmias, and sudden cardiac death. For example, admissions for acute MI were more than double (OR=2.4) in the week after the 1994 Northridge earthquake, as compared with the week before the disaster, especially at hospitals closest to the earthquake's epicenter (54). In addition, Steinberg and colleagues found that there was a significant 2-3 fold increase in implantable cardioverter defibrillator (ICD) therapy in the month following the World Trade Center attacks in 2001 as compared to previous levels (57). There is also evidence that measures of perceived stress are related to onset of new coronary heart disease (58).

Finally, individuals can have different patterns in hemodynamic stress reactivity (i.e., changes in blood pressure and heart rate in response to mental stressors). Research has shown that those with greater blood pressure increases in response to stress may develop higher levels of preclinical cardiac states, including increases in left ventricular mass and enhanced carotid atherosclerosis (59, 60). In addition, greater cardiovascular reactivity in response to mental stress in cardiac patients is predictive of a higher incidence of cardiovascular events (61).

Stress and Heart Failure. The precise importance of psychological variables in heart failure, including stress and mood, is still under investigation. However, several investigators have found evidence that stress and depression

are prevalent in heart failure and may increase symptoms and hospitalizations (62). One group of researchers compared precipitants of hospitalizations between heart failure patients and a non-HF hospitalized control group (9). The results of this study showed that emotional events/stressors (i.e., argument, extreme anxiety, anticipation of dreaded event) precipitated hospitalization more frequently in the heart failure group (49%) than in the control group (24%). Lack of adherence, emotional issues, and environmental issues, along with several physiological measures, were found to be precipitants of decompensation in heart failure in another study (63). In addition, one study found that psychological stress, with hypertension, smoking, and waist to hip ratio, was a significant risk factor for heart failure (64).

Psychosocial stress appears to have an important role in heart failure decompensation and severity. This study will investigate the relationship of these psychological variables with hemodynamic variables that also perform a key role in the process of heart failure. The purpose of examining this relationship in a healthy population is to determine the underlying associations without the complications of other variables related to disease state, in order to inform the research on these concepts in heart failure patients.

### Reactivity (Emotional and Hemodynamic)

Laboratory testing with mental stress and exercise is a useful technique to examine responses to environmental stimuli that may occur during daily life. Although testing in the laboratory setting may seem artificial, mental stress has

been shown to be associated with considerable hemodynamic responses (65). In fact, laboratory levels and reactivity of heart rate and blood pressure to mental stress have shown satisfactory reproducibility with high test-retest correlations and can be generalized to daily life (66, 67). Results of generalizability studies have been inconsistent (68, 69) calling into question the validity of laboratory stressors. However, Kamarck et al. (70) were able to show a robust relationship between laboratory and real-world cardiovascular reactivity using aggregated data from multiple measurements. The authors contend that the methodology of previous studies on generalizability of reactivity were not adequate to obtain consistent results. Evidence from both healthy population and cardiac patient research has shown a relatively consistent pattern of hemodynamic reactivity associated with mental stressors and exercise stressors. Standard aerobic exercise stress is associated with a marked increase in heart rate and cardiac output and a concomitant prominent vasodilation response (65). In general, mental stressors increase heart rate and cardiac output and have vasodilatory effects, but at a lesser magnitude than produced by exercise (65). Comparing mental and exercise stressors, investigators have consistently found that heart rate, cardiac output, and systolic blood pressure increase more with exercise whereas mental stress is associated with greater diastolic blood pressure increases (65, 71, 72).

Greater cardiovascular reactivity in response to mental stress has been associated with greater risk for the development of coronary artery disease, hypertension, and worsening of existing disease (61, 73, 74), although not all

investigators have been able to replicate these findings (75-78). Strike and colleagues found that CAD patients had exaggerated hemodynamic and platelet responses to mental stress as compared with healthy controls (41), which could promote acute coronary events through plaque rupture or thrombogenesis. In addition, heightened hemodynamic reactivity to mental stress testing can identify individuals with CAD who are more likely to experience myocardial ischemia during daily life and with mental stress in the laboratory (74).

### Task-specific Responses

In the present study, cardiovascular reactivity to two types of mental stress will be compared with a particular focus on blood pressure (BP), heart rate (HR), cardiac output (CO), total peripheral resistance (TPR), and brain natriuretic peptide (BNP). The first two tasks are classic mental stressors called mental arithmetic (cognitive) and anger recall speech (emotional). These two tasks have been grouped together because of similar cardiovascular reactivity seen with these tasks. The second type of stressor is a mirror trace task (psychomotor), in which the participant will utilize an apparatus to trace the shape of a star using only their reflection in a mirror. Previous research has found that the pattern of cardiovascular responses differ between these two types of mental stressors (65). The mirror trace task has been described as a strong “vascular” stimulus due to a strong and consistent increase in TPR in response to this task. In contrast, the cognitive and emotional types of mental stressors (such as mental arithmetic and anger recall) can be thought of as weak “cardiac” tasks due to the

greater cardiac output response as opposed to TPR, but this pattern is less consistent across individuals. At relatively low levels of stimulation the increase in cardiac output found in these cognitive/emotional stressors could be due to a decrease in vagal tone at the heart, but at higher levels the increase in cardiac output would be due to increases in cardiac  $\beta$ -adrenergic activity (79).

Situational stereotypy is an important concept to consider for understanding the differences in response patterns between these two types of mental stressors. This concept describes the extent to which different situations elicit stereotypically distinct patterns of physiological responses (80). Lacey (81) explained that the activation process is multidimensional and reflects the intended aim or goal of a behavior. He believes that the interaction of an organism and its environment is key to predicting an arousal pattern (82). Therefore, different types of mental stress tasks may be thought of as requiring varied environmental interactions and as such will produce different physiological responses.

One way to distinguish between tasks is to consider whether “active coping” or “passive coping” is standard for that challenge. Obrist (83) defines active coping as effort to influence or control the outcome of an event and hypothesized that tasks requiring active coping would increase sympathetic activation. In fact, stressors that consist of mental work, active coping, or a defense reaction have a pattern of reactivity that includes increased blood pressure, heart rate, cardiac output, and  $\beta$ -adrenergic activity (83-85). Contextual factors, such as predictability, controllability, competition, and harassment, can

modulate the active coping pattern (79). Mental arithmetic, Stroop color-word test, public speaking, and competitive card games are all tasks that have shown an active coping response pattern (86-89).

On the other hand, stressors that involve aversive vigilance, passive coping, or passive avoidance have a response pattern of increased blood pressure, skeletal muscle vasoconstriction, TPR, and  $\alpha$ -adrenergic activity (79). Given this pattern, it follows that the mirror trace task necessitates passive coping or aversive vigilance, which may explain why that task has shown a strong associated vascular response. These coping strategy-related cardiovascular reactivity patterns provide a plausible rationale for the differences in reactivity between the mental arithmetic and mirror trace tasks to be used in the present study.

#### Rationale for Studying BNP with Mental Stress

Currently, there is little known regarding the effects of mental or emotional stress on BNP levels. However, there is evidence in the human and animal literature that suggests a connection between physiological systems involved in the stress response and BNP. Given the hemodynamic changes known to occur in response to mental stress, it follows that BNP would increase in order to maintain homeostasis and protect the cardiovascular system. The question remains whether changes in BNP levels in response to stress would be evident in a healthy population without heart failure.

A study of rats found that signals for ANP and BNP genes (mRNAs) were observed in the heart at rest and were upregulated in response to immobilization stress reaching a maximum at 1 h (for BNP) and 3 h (for ANP) following the onset of immobilization stress (90). This study provides evidence in an animal model for a possible increase in BNP activity in response to a mental stressor. With regard to other natriuretic peptides, A-type natriuretic peptide (ANP) secretion is more pronounced in panic patients during panic attacks, but is lower at rest in panic patients (91). These results are promising as an insight into the potential for an association between stress and the natriuretic peptide system, especially BNP, which has shown clinical importance in the area of heart failure.

The effects of psychosocial factors on BNP are largely unknown. Although the literature addressing the relationship of mental stress and BNP is scarce, many researchers have investigated the relationship of BNP to physical stress and exercise in particular. The results are inconsistent for healthy participants. There are a number of studies that have shown an increase in BNP in response to exercise whereas others have found no such change in BNP.

One study comparing heart failure patients and healthy controls found that both ANP and BNP increased significantly in response to exercise for the heart failure patients, but there were no significant changes in either hormone for the controls (92). Although ANP was found to be significantly increased in response to exercise in two studies with healthy control subjects, levels of BNP did not change in those studies (93, 94).

On the other hand, there is evidence that exercise can produce a transient increase in BNP, even in healthy individuals. In a study of eight healthy males, plasma ANP was significantly increased during bicycle ( $11.0 \pm 4.5$  to  $37.0 \pm 16.3$ ) and hand-grip exercises ( $14.93 \pm 3.06$  to  $26.49 \pm 3.13$ ) and there was a significant trend for increased plasma BNP levels during both exercises (bicycle:  $1.60 \pm 0.83$  to  $2.48 \pm 1.49$ ; hand-grip:  $1.85 \pm 0.44$  to  $2.40 \pm 0.84$ ) (95). After extreme levels of exercise, such as those from an 100-km ultramarathon, levels of ANP (Pre:  $6.78 \pm 3.26$ ; Post:  $13.54 \pm 7.27$ ) and BNP (Pre:  $3.33 \pm 2.91$ ; Post:  $18.80 \pm 13.33$ ) have been shown to be significantly increased in healthy men (96). But exercise-induced increases in BNP for healthy participants are not limited to extreme exertion levels. Huang and colleagues (97) demonstrated a significant increase in plasma BNP (Pre:  $3.38 \pm 0.50$ ; Post:  $8.21 \pm 2.02$ ; Mean  $\pm$  SEM) immediately after completion of the standard Bruce exercise protocol (98) with 23 healthy young male participants. These investigators also evaluated the time course of BNP from rest to 48 hours post-exercise and found that BNP was only significantly increased immediately after exercise and had returned to resting levels within one hour after exercise (97). In summary, given the hemodynamic reactivity that occurs in response to mental stress, these data on BNP exercise-induced changes may be extrapolated to speculate that mental stress could potentially produce similar BNP changes.

## **Obesity as a Moderator of Stress Responses**

Given the significant role of psychosocial stress in cardiovascular disease, it is useful to investigate how other risk factors interact with cardiovascular stress responses. Obesity has been shown to be important in the initiation (99) and exacerbation (100) of CAD. Therefore, this study will assess the effect of obesity and depression on BNP and other cardiovascular responses to stress. In order to provide an understanding of why these variables may have particular importance, a review of the cardiovascular risk and hemodynamic stress response associated with obesity will follow.

### Definition & Importance for Health Related Concerns

Obesity is a complex phenomenon with biological, social, and psychological causes and consequences (101). Both the CDC and WHO classify obese as having a body mass index (BMI) of  $30 \text{ kg/m}^2$  or greater (102, 103). In the United States, obesity has become an important and growing public health concern. In 2004, 140 million adults in the U.S. were overweight and 66 million were obese (4). Obesity is the second leading cause of preventable death in the U.S., after smoking, with an excess mortality rate of 300,000 deaths per year (104). Health outcomes of chronic and other disease states, such as Type 2 diabetes, cardiovascular disease, hypertension, cancer, and stroke, are all associated with obesity (102). In addition, obesity is a risk factor for cardiovascular disease, diabetes, hypertension, stroke, and cancer (102). The

bottom line is that an individual's level of obesity is positively correlated with their likelihood to develop health problems and accelerate the aging process (105). Therefore, the mechanisms contributing to the excessive health risk associated with obesity are important to investigate, not only due to the health burden, but also because the magnitude of individuals affected by obesity is such a substantial proportion of the population.

Although BMI is the most widely used indicator of obesity, there are other measures of body composition that also confer excess health risk, such as waist circumference, body fat percentage, and waist-to-hip ratio (WHR). WHR is widely used as a measure of fat distribution and is mainly determined by adipose tissue in the abdomen (106, 107). This measure is expressed as a ratio of waist to hip circumference in order to adjust for frame size. Excess abdominal fat is an independent predictor of risk factors and morbidity (108). In particular, WHR has been shown to be associated with an increased risk for diabetes, CAD, and hypertension (109). The higher risk from abdominal fat most likely derives from the enlarged visceral fat deposits that are associated with abdominal obesity (110). Visceral fat is located inside the abdomen between internal organs and subcutaneous fat is found beneath the skin. Visceral fat accumulation is associated with more glucose and lipid metabolism disorders and hypertension as compared with subcutaneous fat accumulation (111). Previous literature suggests that visceral fat has an important impact on CAD risk regardless of obesity (107, 112). Therefore, measures of abdominal adipose tissue, such as

waist circumference and WHR, may provide useful information for disease risk not obtained from BMI.

#### Hemodynamic Stress Reactivity and Obesity

Evidence has shown that obesity is associated with a risky pattern of hemodynamic reactivity in response to mental stress. One study (113) compared a group of insulin resistant (IR) women to a control group of non-insulin resistant women. The IR group had a mean BMI of  $34 \text{ kg/m}^2$ , which falls in the obese category, and the control group had a mean BMI of  $26 \text{ kg/m}^2$ , just above the overweight classification cut-point based on the CDC and WHO guidelines. The results of this study (113) showed that the control group had an increase in CO ( $5.5 \pm 0.8$  to  $6.9 \pm 1.1 \text{ L/min}$ ) and a decrease in SVR ( $1090 \pm 149$  to  $971 \pm 217 \text{ dyn} \cdot \text{s} \cdot \text{cm}^{-5}$ ) in response to mental arithmetic, whereas the IR group had a paradoxical increase in SVR ( $1277 \pm 248$  to  $1397 \pm 218 \text{ dyn} \cdot \text{s} \cdot \text{cm}^{-5}$ ) along with a non-significant increase in CO ( $5.7 \pm 1.3$  to  $6.2 \pm 1.3 \text{ L/min}$ ).

A similar study examined mental stress and handgrip reactivity in male hypertensive participants separated based upon a  $\text{BMI} \geq 27 \text{ kg/m}^2$  and a  $\text{BMI} < 27 \text{ kg/m}^2$  (7). The study, published in 1992, utilized the NHANES 1976-1980 (114) to develop the cutoff point at  $\text{BMI} = 27 \text{ kg/m}^2$ . In the heavier group, these researchers also found a paradoxical and exaggerated increase in TPR with an attenuated increase in CO in response to a mental arithmetic task. Furthermore, the heavier group showed a greater increase in TPR in response to the handgrip task as compared to the leaner group, whereas there was no difference in CO or

stroke volume between the two groups. This study demonstrated that heavier hypertensive individuals respond to a cognitive mental stressor with a less favorable pattern of vasoconstriction rather than the expected vasodilation of the leaner group and exaggerated vasoconstriction in response to isometric handgrip stress (7).

Stress Reactivity in Healthy Obese Individuals. In order to refine the relationship between obesity and hemodynamic reactivity patterns, several additional studies have utilized healthy obese and lean participants without hypertension, diabetes, or insulin-resistance and investigated the relationship with different outcome variables (106, 115, 116). One of these studies investigated the relationship of mental stress reactivity to continuous measures of BMI and waist hip ratio (WHR) in 20 normotensive healthy young men (106). During mental stress, the authors found no significant univariate correlations between BMI and CO or TPR. However, the univariate correlations of WHR with CO and TPR presented a paradoxical pattern similar to previously reported studies, showing a negative correlation with CO ( $r=-0.63$ ,  $p<0.01$ ) and a positive correlation with TPR ( $r=0.49$ ,  $p<0.05$ ). Because BMI and WHR were interrelated in this study, the authors used regression models to assess the relative contribution of each anthropometric index to hemodynamic variables. These analyses revealed the same patterns for WHR with CO and TPR as with the univariate correlations, whereas BMI was found to be positively related to CO and negatively related to TPR. The results from this study suggested a CO dependent pressor response to mental stress associated with higher BMI and

peripheral vasoconstriction associated with higher WHR (106). However, it should be noted that the BMI range for this study was 18.5 – 30.2 kg/m<sup>2</sup>, indicating that this sample was mostly lean and overweight and may not be representative of the patterns that exist in healthy obese individuals. Most importantly, this study highlights WHR, mainly determined by abdominal adipose tissue, as an important index in the classification of dysfunctional hemodynamic reactivity and appears to be a useful marker even below the traditional obesity threshold.

In another study, 23 obese and 23 lean normotensive individuals underwent a mental arithmetic stressor while forearm and skin blood flow responses were measured (116). Blood pressure and TPR are regulated in part by both skin and muscle vascular resistance (117). The investigators of the study hypothesized that the exaggerated blood pressure response to stress associated with obesity might be related to deficient skin and muscle vasodilatation (116). In fact, in the obese group there was a significantly blunted response of forearm vascular resistance and skin microcirculatory dilation as compared to the lean group. These results provide evidence that both skin and muscle vasodilatation in response to mental stress are deficient in obesity. The fact that endothelial dysfunction occurs in forearm resistance vessels with obesity (118) may lead to defects in mental stress-induced vasodilatation. This relationship of endothelial dysfunction and obesity may be an underlying mechanism for the development of hypertension in obese individuals (116).

### The Obesity BNP Handicap & Possible Explanations/Pathways.

The natriuretic peptide system plays a key role in the regulation of renal sodium and water retention, renin-angiotensin, and sympathetic nervous system processes, which are mechanisms linking obesity to the development of hypertension (8). There is speculation that obese individuals have an impaired natriuretic peptide response, described as a *natriuretic handicap* (119). One study, conducted in over 3,000 participants from the Framingham Study, compared baseline levels of BNP and ANP in obese, overweight, and lean individuals (8). The researchers found a decrease in BNP levels with increasing BMI category, such that there was a 1.43 & 1.64 fold increase in the odds of having low BNP for the overweight women and men (respectively) and a 1.84 & 2.51 fold increase in the odds of having low BNP for the obese women and men (respectively). A similar pattern of results was found for the association between ANP and obesity. This difference in ANP and BNP levels supports the *natriuretic handicap* phenomenon (119) and there are several potential mechanisms that may explain the reduced natriuretic peptide levels in obese individuals. First, it has been suggested that adipocytes, the cells that largely compose adipose tissue, participate in the removal of natriuretic peptides from the circulation due to the abundance of natriuretic peptide clearance receptors (NPR-C) in adipose tissue (120). In addition to a greater amount of adipose tissue found in obese individuals, adipose tissue in obese and hypertensive individuals has shown

elevated NPR-C gene expression (119). Therefore, it is possible that those with obesity are able to produce natriuretic peptides at comparable levels to those with a normal BMI, but natriuretic peptides are removed from the system at a faster rate in those with obesity.

Another possible explanation for the natriuretic peptide handicap in obese individuals is that there is dysfunction in the synthesis or secretion/release of natriuretic peptides associated with obesity (121, 122). These two proposed hypotheses are not necessarily mutually exclusive from each other, but further research is needed to illuminate the contribution of each. The present study is designed in part to investigate the relative importance of these suggested pathways.

Lastly, the cross-sectional nature of prior research does not rule out a bi-directional causation model. It is possible that the obese state is a consequence of reduced natriuretic peptide levels. Evidence shows that binding of ANP to NPR-A receptors on adipocytes induces lipolysis, the metabolic process of breaking down lipids to release free fatty acids (123). Lower natriuretic peptide levels would therefore result in reduced lipolysis, which could produce and/or maintain the obese state (8).

### Possible Moderating Effect of Obesity on Hemodynamic Stress Responses

Given the notable relationships between obesity and mental stress hemodynamic responses and the possible explanations for the obesity-related “natriuretic peptide handicap,” this study will examine these two lines of research

together. The importance of the obesity group differences in reactivity is demonstrated by data from Gregg and colleagues (124) that suggests a vascular or mixed hemodynamic reaction to mental stress may pose excess risk for cardiovascular disease. In addition, the reactivity of BNP to mental stress in a healthy sample may also inform heart disease risk, perhaps even more so in the context of obesity-related disparity in BNP levels or action.

Based on the differential hemodynamic reactivity patterns associated with psychomotor (passive coping) and cognitive/emotional (active coping) mental stressors, the model for this study (Figure 2) suggests that the vascular nature of response to the mirror trace task will induce greater BNP increases as compared with the math/speech tasks. As seen in Figures 3 & 4 from Norton (3), TPR affects both preload and afterload, which are associated with myocardial wall stress. Therefore, a task that induces a significant increase in TPR, such as the mirror trace task, will likely be associated with preload and afterload increases, resulting in greater wall stress than a task that has no change or a reduction in TPR. It is known that BNP is released in response to volume overload and wall stress, so it follows that significant increases in TPR will produce a significant increase in BNP.

In this study, TPR reactivity is thought to be a critical factor for BNP release in response to mental stress. The fact that obese individuals respond to mental stressors with a pattern of increased and/or exaggerated TPR as opposed to lean individuals who show a pattern consistent with vasodilation, leads to the conclusion that obese individuals will have similar or greater BNP

increases with both mental stressors despite evidence of lower resting levels of BNP. It is hypothesized for this study that the adipose tissue clearance mechanism explains the obesity “natriuretic peptide handicap” phenomenon completely or to a greater extent than the possibility of dysfunction in production or release of natriuretic peptides. It follows that if there is no difference between obese and non-obese groups in release of BNP, then reactivity of BNP to either type of stressor should be at least comparable and perhaps heightened in the obese group due to mental stress-induced vasoconstriction.

### **Summary and Rationale for the Proposed Study**

Heart failure, and cardiovascular disease in general, pose a substantial public health concern. This review provided evidence that psychosocial stress is related to incidence of coronary disease and cardiovascular events and obesity is a significant risk factor and can change the hemodynamic stress response. In addition, the benefits and potential usefulness for risk stratification of a novel biomarker such as BNP were reviewed. In the present study, we will evaluate the inter-relationships of the important psychosocial variables of stress and obesity utilizing BNP in comparison with more traditional cardiovascular markers of the stress response. The purpose of this investigation is to better understand the physiological consequences of stress with a focus on important risk variable group differences.



## Specific Aims

1. To examine and compare the effects of two acute laboratory mental stressors known to elicit different hemodynamic reactivity patterns in a healthy sample and investigate the role of cardiac output and total peripheral resistance on B-type natriuretic peptide responses to stress.
2. To assess the relationship of obesity to baseline and stress levels of cardiac output, total peripheral resistance, and B-type natriuretic peptide.

## Hypotheses

- Hypothesis I
- (a) Mental stress will be associated with a significant increase in hemodynamic parameters (HR, SBP, DBP) for both types of stress.
  - (b) The mirror trace mental stress task (psychomotor) will not increase cardiac output, but will increase total peripheral resistance.
  - (c) The mental arithmetic and anger recall speech mental stress tasks (cognitive/emotional) will increase cardiac output.
  - (d) Mental stress and exercise tasks will be associated with significant increases in BNP, with similar BNP reactivity

between mental stressors and exercise and greater BNP increase with the mirror trace task as compared to the cognitive/emotional tasks.

Hypothesis II (a) Greater extent of obesity will be associated with greater TPR and reduced CO responses to both types of mental stress.

(b) Greater extent of obesity will be associated with similar or greater BNP responses to the mental stress tasks. A *secondary hypothesis* is that greater extent of obesity will be associated with lower baseline levels of BNP.

## Methods

### Overview

The present research is a laboratory study assessing cardiovascular responses to two types of mental stress and exercise in participants who were either obese, overweight, or had a BMI in the normal range. Obesity status and type of task (cognitive/emotional vs. mirror trace vs. exercise) constituted the primary independent variables. Secondary independent variables of interest included other measures of body composition, such as waist to hip ratio (WHR), fat free mass, and body fat percentage. Physiological measures during rest and both mental stressors represented the primary dependent variables and included: systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), cardiac output (CO), total peripheral resistance (TPR), and B-type natriuretic peptide (BNP). In addition, self-rated mood was evaluated over the course of the study to determine how salient the mental stressors were.

### Participants

Thirty-six participants were consented for this study, but three were excluded because of hypertensive baseline blood pressure readings. Participants were recruited from the Washington, D.C., metro area from newspaper and online resources, including craigslist.com and the Washington City Paper. In addition, flyers were posted on bulletin boards at USUHS. Potential participants were screened over the phone by the Principal Investigator to determine eligibility

for the study based on inclusion and exclusion criteria. Inclusion criteria: Healthy individuals age 18-40 were eligible to participate. Participants were recruited into one of three groups: obese ( $\text{BMI} \geq 30 \text{ kg/m}^2$ ), overweight ( $25 \leq \text{BMI} < 30 \text{ kg/m}^2$ ), and normal weight ( $18.5 \leq \text{BMI} < 25 \text{ kg/m}^2$ ). To participate in both the mental stress and exercise tasks, individuals were required to be military members, military beneficiaries, or Federal employee civilians. Exclusion criteria included: (1) cardiovascular disease, (2) hypertension (blood pressure  $> 140/90 \text{ mmHg}$ ), (3) chronic illness (pulmonary disease, thyroid disorder, renal disease, or liver disease), (4) diabetes, (5) pain/discomfort that may be due to myocardial ischemia, (6) pregnant, (7) cognitive impairments interfering with decision-making, consent, or other study requirements, (8) do not meet BMI category requirements, (9) taking antidepressant medication, (10) allergy to adhesive/tape needed for impedance measurement, (11) inability to travel to study location in Bethesda, MD, and (12) other physical illness that precludes participation in research or the tasks involved for this study (i.e., arthritis or injury). Participants who completed both the mental stress and exercise portions of the study had these additional exclusion criteria: (1) not sufficiently healthy for physical activity based on PAR-Q or physician's assessment (including dizziness, syncope, dyspnea, fatigue, palpitations, tachycardia, chest pain, heart murmur, edema, musculoskeletal problems), (2) claustrophobia. If an individual fulfilled the screening requirements, then they were invited to participate in the study assuming eligibility was met at the research visit (i.e., self-reported weight/height similar to laboratory tested values).

## Procedure

A timeline of the study is presented in Figure 5. At the beginning of the research visit, thorough informed consent was obtained. Measurements of height, weight, and body composition were taken. Weight was obtained on a standard scale and height measured with a standard ruler, in order to calculate body mass index. Waist-hip ratio was assessed using a measuring tape to determine the circumference of the hips at the widest part of the buttocks and the waist at the smaller circumference of the natural waist, usually just above the belly button. To calculate the ratio, the waist measurement was divided by the hip measurement. Body fat percentage was estimated at the beginning of the study by bioelectric impedance (BI). Bioelectric impedance involved placing electrodes on the right hand and foot and then passing a small current through the participant, to obtain resistance of the signal through the body. If the participant's height and weight measurements were considerably different from what was reported during the phone screening, then they were placed into a different BMI category group, if appropriate. Next, two baseline blood pressure measurements were taken and averaged. If the resulting blood pressure was greater than 140/90, then the participant was excluded from the study (based on exclusion criteria) and paid \$10 (as described above).

Next, the primary researcher (S.H.) placed the electrodes for the impedance cardiography on the participant, along with a blood pressure cuff generally on the participant's left arm (unless there was difficulty obtaining

measurements and the cuff needed to be moved to the right arm). All blood pressure, heart rate, and impedance cardiography readings were taken by the primary researcher throughout the study. In addition, an in-dwelling 20-gauge catheter was placed in the participant's arm (generally the right arm unless there were difficulties) by a qualified phlebotomist. During the study, blood was withdrawn from the catheter into tubes by the primary researcher. If a participant experienced pain, swelling, or redness at the site of the catheter, these problems were generally solved by removing the catheter. Alternate latex-free products were used throughout the study, in case a participant had an allergy to latex. However, participants with an allergy to adhesive/tape were determined during the screening and excluded from the study due to the lack of alternate products.

At baseline and in between each task, participants were required to drink water equivalent to 0.17% of their body weight. For a person who weighs 70 kg (154 lbs), the volume would be ~120ml or about 0.5 cups. For a 50 kg person (110 lbs) the volume would be ~85ml or a bit more than 1/3 of a cup. This procedure was used to prevent dehydration. A thirty minute rest period followed during which time the participant were asked to sit alone quietly without falling asleep. During the first rest period, participants filled out a packet of questionnaires including demographics. The questionnaire data are beyond the scope of this dissertation and are not reported here or discussed further. A selection of popular magazines was available to participants during each of the rest periods. The participant was informed that blood pressure and heart rate readings would be taken every 4 minutes throughout the rest period starting at

the 15<sup>th</sup> minute. At the conclusion of the rest period, 10mL of blood was withdrawn from the catheter and placed into tubes containing EDTA (an anticoagulant). This blood draw was the baseline for the first mental stress task.

The participant was then given instructions for the first mental stress task set. The order of tasks was established randomly using a pre-determined computer generated list for each BMI group. At the end of the first mental stress task, 10mL of blood was withdrawn from the catheter and placed into tubes containing EDTA. In addition, a series of Likert scales assessing mood were completed. Afterwards, participants sat quietly again for another 30 minute rest period. Blood pressure and heart rate measurements were taken every 4 minutes during rest starting at the 15<sup>th</sup> minute and 10mL of blood was withdrawn at the conclusion of the rest period as the baseline blood draw for the second mental stress task. The participant then completed the second mental stress task, either cognitive/emotional or psychomotor stress. At the end of the second mental stress task, 10mL of blood was withdrawn from the catheter and placed into tubes containing EDTA, the Likert mood ratings were completed again, and the impedance cardiography electrodes were removed.

### *Mental Stress Tasks*

The cognitive/emotional stress included a combined battery of mental arithmetic and anger recall tasks with mental arithmetic always conducted first and each task conducted for 5 minutes. Both tasks were included in order to reduce practice effects or burn out from conducting either task alone, while

lengthening the stressor to 10 minutes. This amount of time was estimated to be more comparable to the length of the exercise stress test, which would be compared to the mental stressors. During mental arithmetic, a second researcher asked the participants to listen to the instructions for that task, which were pre-recorded and played back on a portable compact disc player. The instructions were to count backward by serial sevens starting with a four digit number and to do so as quickly and accurately as possible. A second researcher provided mild harassment during the task, such as correcting the participant when they make an error and prompting the participant to go faster. A pre-recorded metronome sound was played during completion of this task. The primary researcher did not participate in this task, but was present to ensure cardiovascular measurements recorded properly. Immediately after completion of the mental arithmetic task, and without an intervening rest period, participants were given instructions for the anger recall task by the primary researcher. Participants were asked to give a speech recalling a recent anger-provoking incident and discuss the circumstances of the incident with the primary researcher. During these mental stress tasks, blood pressure and heart rate measurements were taken.

For the psychomotor task, participants completed a frustrating mirror trace task twice, once with their dominant hand and once with their non-dominant hand. If the catheter presented too much discomfort, participants completed the mirror task twice with the same hand. The participant was instructed to trace the outline of a star in an apparatus (BRS/LVE, Beltsville, MD) that recorded the number of errors made (i.e., participant moved the stylus outside the defined

outline). In addition, the apparatus only allowed the participant to watch their movements through their reflection in a mirror. The mirror trace task was conducted for 5 minutes with each hand, during which blood pressure and heart rate measurements will be taken.

### *Exercise Task*

During the third 30-minute rest period, the primary researcher placed the electrodes for a 12-lead electrocardiogram (ECG) and the Polar heart monitor for heart rate. In addition, participants underwent a physician's assessment to determine whether the individual was healthy enough to engage in moderate physical activity. This evaluation supplemented the health information self-reported by participants during the telephone screening, including the Physical Activity Readiness questions (PAR-Q) in order to triangulate on the risk exercise posed to each participant. If the physician's clinical judgment was that the participant should not exercise, then the study would have been stopped and the participant would be compensated for their time. However, all participants eligible for the exercise portion of the study, were deemed healthy and able to complete the exercise task. At the 25<sup>th</sup> minute of the rest period two blood pressure and heart rate readings were taken to be used as the baseline measures for exercise. After the rest period, 10mL of blood were withdrawn from the catheter as a baseline measure for exercise. The primary and secondary researchers then assisted the participant in putting on the face mask, used to measure lung function, just prior to beginning the exercise challenge. If a participant reported

feeling claustrophobic from wearing the face mask, then the exercise test would be terminated, although no participants reported this feeling during the study.

Participants underwent a maximal incremental treadmill test to volitional exhaustion to document their maximal aerobic capacity  $\text{VO}_{2\text{max}}$  and challenge the hypothalamic-pituitary-adrenal (HPA) axis. In brief, this test involved a 5 min warm-up (3.0 mph, 2% slope grade), followed by walking/running on a treadmill (Quinton ST-65, Quinton Instrument Co., Seattle, WA) at 3 to 7 mph (depending on each participant's heart rate 4 minutes into the warm-up) at increasing slope grades (+2.5%) every two minutes until the subject can go no further (approximately 20 minutes), followed by a cool-down period to safely return the participant to a normal heart rate. This test was a modification of the protocol described by Kyle et al. (125), which involves a slightly different warm-up procedure.

Ten mL of blood was obtained before, 5 mL immediately after the final stage, and 5mL 15 min after exercise. Subjects were instrumented with electrodes (Stressvue, Philips, Netherlands) and Polar heart monitor for continuous monitoring of heart rate and ECG and were instrumented with a face mask for measuring oxygen uptake, carbon dioxide production, and respiratory exchange ratio by open circuit spirometry (Oxycon Mobile, VIASYS Healthcare Inc.). With open circuit spirometry, the participant wears a mask that allows them to breathe in ambient air and the exhaled air exits through a gas meter to be measured and analyzed (126). During this exercise challenge, participants were monitored by a physician and a researcher stood by them as a spotter to ensure

they do not slip and fall. The exercise was stopped when the participant requested (volitional exhaustion). Participants completed the mood Likert scales immediately before and immediately after the exercise challenge. In addition, two blood pressure and heart rate measurements were taken immediately after the participant completed the treadmill test and returned to a seated position.

### *Post-Stress*

After completion of all mental stress tasks and the exercise test (when conducted), the in-dwelling catheter, electrodes, and blood pressure cuff were removed. The researcher debriefed the participants about the objectives of the study, explained why they were hassled during the mental arithmetic task, answered any questions the participant had, and collected information to compensate the participant. After making sure the participant was feeling well, the individual's participation was complete and participants received compensation by mail.

### Measures

#### *a) Screening Questionnaire*

In order to qualify to participate in this study, potential candidates were screened over the phone using this questionnaire (Appendix) to assess for inclusion and exclusion criteria. This measure included a brief medical history portion about conditions such as diabetes, cardiovascular disease, hypertension, cognitive impairments, and other physical illnesses that could interfere in

participation. In addition, potential participants self-reported their age, weight, and height to determine eligibility.

b) *Demographics*

Variables measured include: age, sex, ethnicity, years of education, marital status, living arrangements, and smoking status. Participants completed this questionnaire at the beginning of the study and the information was used in analyses where appropriate or necessary.

c) *Body Mass Index*

Participants had their height and weight measured with a standard scale and measuring device at the beginning of the study to confirm BMI eligibility. Body mass index (BMI) is defined as weight in kilograms divided by height in meters squared ( $\text{kg/m}^2$ ). Both the Center for Disease Control and the World Health Organization classify a  $\text{BMI} \geq 30 \text{ kg/m}^2$  as obese,  $25 \leq \text{BMI} < 30 \text{ kg/m}^2$  as overweight, and  $18.5 \leq \text{BMI} < 25 \text{ kg/m}^2$  as the normal range (102, 103). Although distribution of fat may be a more accurate measure of risk stratification, BMI was used as the primary variable in this study for group classification purposes because it has been used in previous research on the natriuretic peptide handicap and it is a standardized and effective way to categorize participants, especially during a telephone screening. However, during the study, measurements of waist circumference, waist-to-hip ratio, fat free mass, and body fat percentage were collected to use in exploratory analyses. Magnetic resonance imaging (127) and computed tomography (128) can provide accurate assessment of abdominal fat, although these techniques are not practical for

routine use in research or clinical practice. Waist circumference and waist-to-hip ratio measurement of abdominal fat correlates with these advanced measures and can be useful as a marker of abdominal obesity (108). Bioelectric impedance was also used to obtain measures of fat free mass (total mass minus fat) and body fat percentage (weight of fat mass divided by body weight) using standard equations from NHANES III (129).

d) *Blood Pressure & Heart Rate*

Systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) were assessed throughout the study as measures of hemodynamic baseline and reactivity levels. A Critikon Dinamap automated BP monitor and cuff were used to take measures of blood pressure and heart rate. During the rest periods, BP and HR were measured every 4 minutes, starting at the 15<sup>th</sup> minute. Baseline measures of BP and HR were calculated as the mean of the measurements taken during the rest period. BP and HR were captured at 30 seconds, 1 minute 30 seconds, and 2 minutes 30 seconds during each 5-minute mental stress task. Mean BP and HR of each task were used for mental stress measures. Change from baseline to mean mental stress levels were used to evaluate hemodynamic reactivity to each task. This method directly utilized the raw data and has been described as comparable in reliability and outcome with other approaches to calculating reactivity scores (130, 131).

e) *Impedance Cardiography (CO & TPR)*

Impedance cardiography is a non-invasive measure of resistance to transmission of a small electrical current throughout the body (132). This

technique has been used to examine cardiovascular responses in clinical and laboratory settings (133). The impedance cardiography technique utilizes a pair of electrodes that introduce a small electrical current to the skin and another pair of electrodes that receive information on resistance. These electrodes are placed on the neck and chest in order to pass the small electrical current across the chest (134). Hemodynamic measures are either directly recorded by the impedance device or indirectly calculated through validated algorithms based on the data that is collected (132). Changes in impedance allow for calculation of stroke volume with either Kubicek's equation (135) or the equation derived by Sramek (136). Cardiac output and total peripheral resistance can then be derived using stroke volume, ventricular ejection time, and mean arterial pressure (134). In the present study, using the Kubicek equation (135), the dependent variables of cardiac output and total peripheral resistance were calculated via algorithms integrated into the impedance device after input of blood pressure data.

Although the "gold standard" in CO & TPR measurement is considered to be with a pulmonary artery catheter method, that technique is invasive and safety concerns have been raised (137). Certainly, the risks associated with using pulmonary artery catheters outweighed the benefits for use in the present observational research study. Fortunately, the non-invasive technique of impedance cardiography has been shown to be a safe and reliable alternative to invasive measures (133).

f) *B-Type Natriuretic Peptide*

Each blood sample was collected in tubes containing EDTA, spun within 1 hour of collection, and frozen at -70°F. Samples were analyzed for BNP at the University of Maryland Medical Center. Frozen samples were thawed in a room temperature water bath, vortexed to mix, and then spun cold at 5°C for 10 minutes at 28000rpm to remove particulate matter. The resulting plasma was used to test BNP on the Beckman Access 2 instrument with the Triage BNP test. All samples were de-identified and blinded to groups and tasks for the laboratory personnel.

BNP was assessed via fluorescence immunoassay for the quantitative determination of BNP in whole blood and plasma specimens in which EDTA is the anticoagulant. After addition of a blood sample to the sample port of the test device, the red blood cells are separated from the plasma via a filter. A predetermined quantity of plasma moves by capillary action into a reaction chamber and is allowed to react with fluorescent antibody conjugates within the reaction chamber to form a reaction mixture. After an incubation period, the reaction mixture flows through the device detection lane. Complexes of the analyte and fluorescent antibody conjugates are captured on discrete zones in the detection lane. Excess plasma sample washes the unbound fluorescent antibody conjugates from the detection lane into a waste reservoir. The concentration of the analyte in the specimen is proportional to the fluorescence bound to the detection lane.

#### g) *Mood Ratings*

Participants scored their baseline and post-stress emotions on a 7-point Likert scale (Appendix). The questionnaire asked how the participant was feeling

“right now” on each of the 7 emotions, which included “anxious, frustrated, irritated, tired, challenged, tense, sad, interested, and angry.”

### Statistical Analyses and Power Calculations

Demographic comparisons between the weight groups utilize ANOVAs for continuous variables and logistic regression for dichotomous variables.

Hypothesis-specific analyses were conducted as follows:

Hypothesis I(a): Mental stress will be associated with a significant increase in hemodynamic parameters (HR, SBP, DBP) for both types of stress.

*Analyses*: For both mental stress tasks, paired samples *t* tests were conducted on each hemodynamic reactivity measure (HR, SBP, DBP, CO, TPR) and BNP to assess change from baseline to mean stress level, after exploring the overall main effects and interactions with two-way within-subjects repeated measures ANOVAs (time x task).

*Power calculation*: A power analysis (138) was conducted to determine the sample size necessary for this hypothesis based on previous research into hemodynamic responses to mental stress (65) with effect size indexes of  $d_z=0.65-1.48$ . The smallest of these effect sizes required 21 participants to detect within-group differences using  $\alpha=0.05$  and 80% power.

Hypothesis I(b) and (c): Mirror trace mental stress task will not increase cardiac output, but will increase total peripheral resistance. Cognitive/emotional mental stress tasks will increase cardiac output.

*Analyses:* Two-way (time x task) repeated measures ANOVAs were conducted to assess CO and TPR reactivity to the mental stressors and examine whether reactivity differed by mental stress task (cognitive/emotional versus mirror trace).

*Power calculation:* A power analysis (138) was conducted to determine the sample size necessary for these hypotheses based on previous research (65, 113) with effect size indexes of  $d_z=0.76-1.03$ . The smallest of these effect sizes requires 16 participants to detect within-group differences using  $\alpha=0.05$  and 80% power.

Hypothesis I(d): Mental stress and exercise tasks will be associated with significant increases in BNP, with similar BNP reactivity between mental stressors and exercise and greater BNP increase with the mirror trace task as compared to the cognitive/emotional tasks.

*Analyses:* Two-way (time x task) repeated measures ANOVAs were conducted to determine whether BNP increased significantly in response to the stressors and if there were task differences in BNP responses.

*Power calculation:* A power analysis (138) was conducted to determine the sample size necessary for this hypothesis based on previous research into BNP response to exercise (97) with an effect size index of  $d_z=0.48$ . This effect size requires 36 participants to detect within group differences using  $\alpha=0.05$  and 80% power.

Hypothesis II(a): Greater obesity will be associated with greater TPR and reduced CO responses to both mental stress tasks.

*Analyses:* Three-way (time x task x weight groups) repeated measures ANOVAs were conducted to examine if the change in CO and TPR differs by weight group. Mental stress tasks were also analyzed separately as the patterns of change for each task were expected to differ regardless of weight group.

*Power calculation:* A power analysis (138) was conducted to determine the sample size necessary for this hypothesis based on previous research correlating TPR and CO responses to body fat (106) with effect sizes of  $r=0.49-0.63$ . The smallest of these effect sizes requires 27 participants to detect between-group differences using  $\alpha=0.05$  and 80% power.

Hypothesis II(b): Greater obesity will be associated with similar or greater BNP responses to the mental stress tasks. A *secondary hypothesis* is that greater obesity will be associated with lower baseline levels of BNP.

*Analyses:* Three-way (time x task x weight group) repeated measures ANOVAs also were conducted to compare BNP responses between weight groups on the mental stress tasks. Weight groups were compared on baseline dependent variables using one-way ANOVAs for continuous measures and logistic regression for dichotomous measures.

*Power calculation:* A power analysis (138) was conducted to determine the sample size necessary for the main hypothesis using an effect size of  $d=0.99$ , obtained from previous research on exercise-induced ANP response in obese women (139), which was the only available study with relevant results for sample size estimation. This effect size requires 10 participants to detect a within group difference in the obese group using  $\alpha=0.05$  and 80% power. It is unclear how

many participants are required to detect a between-groups difference, but perhaps tripling the participants from the obese data could be sufficient, for a total of 30 participants (10 in each weight group).

A separate power analysis (138) was conducted for the secondary analysis. Based on previous research (8, 140), the sample size required for the associated effect sizes ( $d=0.34-0.41$ ) would be between 190 and 272 total participants, which is not feasible for the present study. A study conducted in heart failure patients did find a strong effect ( $d=4.39$ ) for significantly lower BNP levels in obese versus lean individuals (141). Because this finding was with heart failure patients, it was unclear whether this robust result would replicate in a non-CHF sample. Therefore, this hypothesis was explored as a secondary analysis because it was unlikely that this study would be adequately powered to detect a significant difference in a healthy sample.

## Results

### Sample Characteristics

Of the 36 participants consented for this study, three were excluded because of hypertensive baseline blood pressure readings (BP > 140/90). Therefore, the total N for this sample was 33, including 18 in the normal weight group, 5 in the overweight group, and 10 in the obese group. The sample characteristics by weight groups are presented in Table 2. As expected, BMI was significantly higher in the overweight and obese groups than in the normal weight group, as well as being significantly higher in the obese versus overweight groups ( $F_{2,30}=101.54$ ,  $p<0.002$ ). Although the weight groups were comparable in age and years of education, the obese group consisted of significantly more African Americans than the normal weight group (OR=8.13,  $p<0.04$ ) and the overweight group consisted of significantly fewer females than the normal weight or obese groups (OR=0.07,  $p<0.05$  & OR=0.06,  $p<0.05$ , respectively). In addition, the obese group had significantly higher baseline SBP ( $F_{2,30}=4.77$ ,  $p<0.02$ ) and marginally higher DBP ( $F_{2,30}=3.19$ ,  $p=0.055$ ) as compared to the normal weight group. Although a few more individuals completed the mirror task ( $n=18$ ) before the math/speech task ( $n=15$ ), there were no significant differences in physiological measure changes between these two groups.

Sample size for this study was estimated using power calculations based on effect sizes from previous research. Analyses were also conducted after data collection to determine the effect sizes found for each hypothesis and the power

observed in this study. The data from these effect size and power calculations can be found in Table 3.

### Specific Aim 1

Specific Aim 1 concerned the effects of two acute laboratory mental stressors (cognitive/emotional vs. psychomotor) on hemodynamic reactivity patterns and B-type natriuretic peptide responses. The hypothesis for this aim was divided into four parts: (a) mental stress will be associated with a significant increases in hemodynamic parameters (HR, SBP, DBP) for both tasks; (b) the mirror trace task will not increase cardiac output, but will increase total peripheral resistance; (c) the cognitive/emotional tasks will increase cardiac output; (d) the mental stress and exercise tasks will be associated with significant increases in BNP, with similar BNP reactivity between mental stressors and exercise and greater BNP increase with the mirror trace task as compared to the cognitive/emotional tasks.

### Hypothesis 1

(a) The first part of Hypothesis 1 dealt with mental stress hemodynamic reactivity. Results of paired-samples *t* tests indicated that SBP, DBP, and HR significantly increased in response to both the cognitive/emotional tasks and the mirror task as expected. CO was significantly increased in response to the cognitive/emotional tasks and significantly decreased in response to the mirror task. TPR showed no change in response to the cognitive/emotional tasks, but

significantly increased in response to the mirror task. Mean responses to the tasks (stress-rest),  $t$  values, and  $p$  levels for these variables can be found in Table 4. For both the cognitive/emotional and mirror stressors, change in CO was negatively correlated with change in TPR ( $r=-0.48$ ,  $p<0.006$ ;  $r=-0.47$ ,  $p<0.008$ , respectively; see Figure 6).

To assess whether the two types of tasks were effective stressors, mood ratings completed immediately before and after each task were compared. The cognitive/emotional tasks significantly increased mood ratings of anxious, frustrated, irritated, challenged, tense, and angry. The mirror task significantly increased mood ratings of anxious, frustrated, irritated, tired, challenged, and tense. These changes were still significant after adjusting for the nine comparisons made in each task ( $\alpha=0.05/9$ ). Mood ratings that were significantly increased for each task were correlated with changes in the physiological measures. For the cognitive/emotional tasks, irritation correlated with HR and CO ( $r=0.05$ ,  $p<0.004$  &  $r=0.39$ ,  $p<0.03$ ), challenge correlated with BNP ( $r=0.49$ ,  $p<0.006$ ), and angry correlated with DBP ( $r=-0.40$ ,  $p<0.03$ ). For the mirror task, tired correlated with SBP ( $r=-0.43$ ,  $p<0.02$ ) and frustration correlated with DBP ( $r=-0.43$ ,  $p<0.02$ ).

(b) & (c) These hypotheses involve the comparisons of hemodynamic responses to the two types of mental stress. Comparisons of CO and TPR responses to the mental stress tasks were analyzed via two-way repeated measures ANOVAs. Mental stress reactivity of CO showed a significant

interaction of time and task ( $F_{1,31}=51.51, p<0.001$ ), such that the cognitive/emotional tasks showed a greater increase in CO as compared to the mirror task (Figure 7). Similarly, mental stress TPR reactivity showed a significant interaction of time and task ( $F_{1,31}=22.59, p<0.001$ ), with a greater increase in TPR produced by the mirror task versus the cognitive/emotional tasks (Figure 8). When comparing the tasks on hemodynamic responses, interactions of time and task were found for SBP and DBP, with the cognitive/emotional tasks increasing SBP somewhat more than the mirror task ( $F_{1,32}=3.35, p=0.077$ ). The cognitive/emotional tasks also increased DBP significantly more than the mirror task ( $F_{1,32}=6.60, p<0.02$ ). A summary of the repeated measures ANOVAs for these five cardiovascular measures can be found in Table 5.

(d) This hypothesis involves BNP reactivity to mental stress tasks and exercise. BNP values in young, healthy individuals are relatively low and therefore do not follow a normal distribution (Figure 9). Due to the high degree of positive skewness on the BNP values in this sample (Skewness coefficients=1.61-2.00, S.E.<sub>skew</sub>=0.41), analyses of BNP were conducted on natural log transformations of the data. Other investigators who have researched BNP levels in healthy individuals have also transformed the data in a similar fashion (142).

Raw values and natural log transformed values for BNP at each time point are displayed in Table 6. BNP increased significantly in response to both mental stressors (Cog:  $t_{31}=-3.24, p<0.004$ ; Mirror:  $t_{31}=-2.50, p<0.02$ ) and exercise ( $t_7=-$

15.52,  $p < 0.001$ ). The percent increase in log BNP from resting levels was approximately 3-21% with mental stress and 38% with exercise. A comparison of BNP responses to all three tasks showed a significant interaction of time and task ( $F_{2,14} = 155.66$ ,  $p < 0.001$ ) with BNP increasing significantly more in response to exercise as compared to the mental stressors (Figure 10). In addition, BNP measured 15 minutes after exercise was analyzed in comparison to baseline for exercise, peak exercise, and mental stress tasks. Although BNP decreased significantly from peak exercise to 15 minutes post exercise ( $F_{1,7} = 332.28$ ,  $p < 0.001$ ), it remained significantly higher than at exercise baseline ( $F_{1,7} = 39.34$ ,  $p < 0.001$ ). The comparison of BNP responses to the mental stress tasks and 15-min post-exercise found an interaction of time and task ( $F_{2,14} = 17.97$ ,  $p < 0.001$ ) with BNP remaining significantly increased 15 minutes after exercise as compared to the increase in either mental stress task.

There was no significant interaction of time and task when comparing BNP responses to the two mental stress tasks only ( $F_{1,30} = 0.48$ ,  $p = 0.50$ ). In addition, no difference was found between BNP levels for the first and second rest periods ( $t_{30} = -0.23$ ,  $p = 0.82$ ) indicating that BNP returned to baseline by the second rest measure, which occurred 30 minutes after the first stressor.

#### BNP in Relation to Age and Sex

Previous research has shown that BNP varies with both age and sex (142-144). Therefore, age and sex were assessed to determine if either was related to BNP in the present sample. There was no association of age (either continuous

or categorical) with BNP values in this sample, perhaps due to the restricted age range included in this study. In contrast, sex was related to BNP levels at all 4 mental stress time points, such that females had higher levels of BNP than males ( $p$ 's<0.03). BNP levels were higher in females than males before and after exercise, however these analyses were not significant due to reduced sample size for the exercise task ( $N=8$ ) and subsequent lack of power for these analyses. Therefore, BNP responses to all three tasks were reanalyzed using sex as a between-subjects factor. For both mental stress tasks (Cog:  $F_{1,30}=5.53$ ,  $p<0.03$ ; Mirror:  $F_{1,30}=6.02$ ,  $p<0.03$ ) and exercise ( $F_{1,6}=319.91$ ,  $p<0.001$ ) there remained a significant increase in BNP. In addition, there was a significant interaction of time and sex for BNP response to the cognitive/emotional tasks ( $F_{1,30}=5.00$ ,  $p<0.04$ ) with females increasing more from pre to post-task than males (Figure 11). The comparison of BNP responses to all three tasks found an interaction between time and task ( $F_{2,12}=166.70$ ,  $p<0.001$ ) such that exercise increased BNP significantly more than the mental stress tasks. The interaction of time, task (3 levels), and sex on BNP was not significant ( $F_{2,12}=1.73$ ,  $p=0.22$ ). As with the analysis of peak exercise response, there was no interaction of time, task, and sex ( $F_{2,12}=1.45$ ,  $p=0.27$ ) for 15-min post-exercise, although the interaction of time and task remained significant ( $F_{2,12}=18.77$ ,  $p<0.001$ ).

Examining BNP responses to the two mental stress tasks by sex revealed that the interaction of time and task remained non-significant ( $F_{1,29}=0.30$ ,  $p=0.59$ ), but there was a three-way interaction of time, task, and sex ( $F_{1,29}=13.85$ ,  $p<0.002$ ). For females, BNP increased more with the cognitive/emotional tasks

than the mirror task, but for males, BNP increased more for the mirror task than the cognitive/emotional tasks (Figure 12).

#### Correlations Among BNP, CO, and TPR

Although the hypotheses did not explicitly address possible associations between the changes in hemodynamic measures and BNP, it was thought that the mirror trace task would increase TPR more than the cognitive/emotional tasks and that would in turn be related to a greater increase in BNP. Correlations were conducted to determine if such an association existed. There were no significant correlations between the changes in CO, TPR, and BNP in response to either task. However, there were positive correlations between BNP and CO levels at stress for the cognitive/emotional and mirror tasks ( $r=0.48$ ,  $p<0.007$  &  $r=0.37$ ,  $p<0.04$  respectively). These correlations were also examined by sex because of sex differences for these variables. The relationship of BNP and CO levels at stress remained significant in females for the cognitive/emotional tasks ( $r=0.44$ ,  $p<0.05$ ), but not for males nor in either sex for the mirror task.

#### Specific Aim 2

Specific Aim 2 was to assess the relationship of obesity to baseline and mental stress reactivity of hemodynamic measures and BNP. The hypothesis for this aim was divided into two main parts: (a) greater obesity will be associated with greater TPR and reduced CO responses to both mental stressors; (b) greater obesity will be associated with similar or greater BNP responses to the

mental stress tasks and greater obesity will be associated with lower baseline levels of BNP.

## Hypothesis 2

(a) The first hypothesis for this aim dealt with hemodynamic reactivity differences across the weight groups. Results indicated that the three weight groups increased similarly in heart rate and blood pressure to the cognitive/emotional tasks. For the mirror task, there was a significant interaction of time and weight group for DBP ( $F_{2,30}=3.49$ ,  $p<0.05$ ) and HR ( $F_{2,30}=5.39$ ,  $p<0.02$ ). For both measures, the normal weight group increased significantly more than the obese group ( $p<0.02$ ,  $p<0.003$ ). Resting values of DBP were also significantly higher in the obese and overweight group than the normal weight group for the cognitive/emotional task rest period ( $F_{2,30}=5.37$ ,  $p<0.02$ ) and higher in the obese group than the normal group for the mirror task rest period ( $F_{2,30}=6.06$ ,  $p<0.007$ ). There were no significant differences between the weight groups on resting values of SBP or HR.

Significant differences between the weight groups were found for CO and TPR values at rest and at stress for both mental stressors (Tables 7 & 8). Due to the inconsistent evidence surrounding the validity of absolute values of stroke volume and CO from impedance cardiography, particularly with overweight individuals (145-148), the focus of these analyses will remain on change of CO and TPR from rest to stress. For CO and TPR, the effect of time for both mental stressors was not changed by the addition of weight groups as a between-

subjects factor. All three weight groups had similar increases in CO to both stressors. TPR response to the cognitive/emotional tasks did show a marginally significant interaction between time and weight group ( $F_{2,29}=3.16$ ,  $p=0.058$ ). The obese group showed a TPR decrease in response to the cognitive/emotional task whereas the other two weight groups showed no change or a slight increase in TPR (Figure 13). The addition of weight groups to the ANOVA equation also did not affect the relationship of time and task for CO or TPR. Although there was a marginally significant three-way interaction of time, task, and weight group for TPR ( $F_{2,29}=2.73$ ,  $p=0.08$ ), in which the differences in TPR response to the cognitive/emotional tasks described above are emphasized (Figure 14). Within each weight group, the interaction of time and task on TPR was significant.

#### Other Body Composition Measures

Exploratory analyses were conducted to examine the relationship of other body composition measures (waist circumference, waist to hip ratio, fat free mass, and body fat percentage) to CO & TPR as compared with BMI and BMI-derived weight groups. Among the continuous body composition measures, including BMI, waist to hip ratio (WHR) was the only index to show a significant relationship with CO and TPR changes to mental stressors. WHR was negatively correlated with change in CO in response to the cognitive/emotional tasks ( $r=-0.39$ ,  $p<0.03$ ) and positively correlated with change in TPR in response to the mirror task ( $r=0.48$ ,  $p<0.006$ ). There were no significant relationships of WHR with CO change to the mirror task or TPR to the cognitive/emotional task.

Because the ideal WHR is smaller in females versus males, these analyses were re-run separately for males and females to reduce any bias from sex. For males, WHR was negatively correlated with change in TPR in response to the cognitive/emotional tasks ( $r=-0.77$ ,  $p<0.02$ ) and positively correlated with change in TPR in response to the mirror task ( $r=0.70$ ,  $p<0.04$ ). For females, WHR was positively correlated with change in TPR to both type of stress (Cognitive/Emotional:  $r=0.43$ ,  $p<0.04$ ; Mirror:  $r=0.43$ ,  $p<0.04$ ). The correlations of WHR and CO responses to both stressors were no longer significant for either sex, although for females there was a negative correlation of WHR with CO change to the cognitive/emotional tasks that approached significance ( $r=-0.38$ ,  $p=0.07$ ).

(b) The second part of Hypothesis 2 involved the role of obesity with respect to BNP. Results indicated that there were no significant differences between the weight groups on rest or stress levels of BNP. After addition of weight groups as a between-subjects factor, the BNP increase in response to both mental stressors remained significant, but there were no differences by weight groups.

The comparison of BNP responses to the two mental stress tasks by weight groups found a significant interaction between time, task, and weight group ( $F_{2,28}=3.44$ ,  $p<0.05$ ). This result was driven by the relationship of BNP responses between the tasks in the overweight group, which showed a decrease in BNP to the cognitive/emotional tasks and an increase in BNP to the mirror task

(Figure 15). In comparison, the normal weight group and obese group had an increase in BNP to both mental stressors with a somewhat greater increase to the cognitive/emotional tasks versus the mirror task (although not significant). The exercise task was not included in these analyses because almost all of those who completed all three tasks were in the normal weight group.

Because of the relationship of sex and BNP levels, the previous analysis was also repeated with both weight groups and sex as between-subjects factors. The analysis revealed that the interaction of time, task, and weight groups was no longer significant ( $F_{2,25}=1.12$ ,  $p=0.34$ ), but the interaction of time, task, and sex remained significant ( $F_{1,25}=4.95$ ,  $p<0.04$ ). Therefore, this three-way interaction of time, task, and sex was likely due to the fact that there are sex differences in BNP responses to the two tasks and that the overweight group was predominantly male.

#### Other Body Composition Measures and BNP

Correlations were conducted to determine if other body composition measures, apart from weight groups based on BMI, were related to BNP. Waist to hip ratio was found to be negatively correlated with rest and stress levels of BNP and body fat percentage was found to be positively correlated with rest and stress levels of BNP (Table 9). However, both of these body composition measures differ by sex in the general population and in this sample, such that females have lower waist to hip ratios ( $t_{27.5}=4.17$ ,  $p<0.001$ ) and higher body fat percentages ( $t_{31}=-3.69$ ,  $p<0.002$ ).

Separate analyses were conducted to examine the relationship of BNP with WHR and body fat percentage for males and females. For males, there were no significant associations. For females, WHR remained negatively correlated with BNP at rest before both tasks (Cognitive/Emotional Rest:  $r=-0.41$ ,  $p=0.056$ ; Mirror Rest:  $r=-0.43$ ,  $p<0.05$ ), but body fat percentage was not significantly correlated with any BNP measures. In addition, females showed a relationship between change in BNP in response to the mirror task and WHR that approached significance ( $r=0.37$ ,  $p=0.088$ ), but males did not.

#### Natriuretic Handicap and Obesity

Lastly, to evaluate the secondary hypothesis that obese individuals would have a “natriuretic peptide handicap,” the weight groups were compared on BNP from the first blood draw regardless of which task they completed first. There were no significant differences between the weight groups on baseline BNP ( $F_{2,30}=1.01$ ,  $p=0.38$ ) as well as no significant correlation between BMI and baseline BNP ( $r=0.17$ ,  $p=0.36$ ). There were no sex differences in the relationship of weight group to baseline BNP. It should be noted that the weight group comparison on baseline BNP was found to be underpowered ( $1-\beta=21\%$ ), as expected.

Based on the findings described above for WHR, body fat percentage, and BNP, these body composition measures were also evaluated with baseline BNP. There was a negative correlation with baseline BNP for WHR ( $r=-0.48$ ,  $p<0.006$ ) and a positive correlation for body fat percentage ( $r=0.36$ ,  $p<0.05$ ). These

associations were again calculated separately for males and females for the reasons stated earlier. The association of baseline BNP with body fat percentage was no longer significant for either males or females. The correlation of baseline BNP and WHR remained significant for females ( $r=-0.44$ ,  $p<0.04$ ), but not for males ( $r=0.09$ ,  $p=0.80$ ).

## Discussion

### Summary of Results

Cardiovascular Responses. Results from this study confirm that laboratory mental stressors produce significant cardiovascular responses in a young healthy sample. Blood pressure and heart rate increased significantly to both stressors. The expected task-specific response patterns were found, such that the cognitive/emotional tasks elicited a strong cardiac output response and the mirror trace task showed a strong total peripheral resistance increase. A comparison of the tasks on hemodynamic parameters demonstrated that the cognitive/emotional tasks increased cardiac output, SBP, and DBP more than the mirror task. Conversely, total peripheral resistance was increased more with the mirror task as compared to the cognitive/emotional tasks. Heart rate increased similarly for both stressors.

BNP. As predicted, BNP increased significantly in response to each of the three tasks (cognitive/emotional stress, mirror stress, and exercise). However, the increase in BNP with exercise was markedly larger as compared with the mental stressors. The size of log BNP increase with mental stress was approximately 3-21% versus 38% increase with exercise. Even BNP collected 15 minutes after exercise showed a greater increase from baseline in comparison to the mental stress tasks. Therefore, the hypothesis that the mental stressors would produce similar BNP reactivity to exercise was not confirmed. Also

contrary to the hypotheses, there was no difference in BNP reactivity when comparing the two mental stressors.

Weight Groups. These results did not confirm the weight group patterns of CO and TPR from most of the previous literature (7, 113, 115). In fact, there was a difference in TPR responses to the two tasks by weight group that approached significance, but was in the opposite direction as predicted. The obese group decreased in TPR in response to the cognitive/emotional tasks and had a similar TPR response to the mirror task as compared to the other weight groups (Figure 14).

Mental stress response of BNP was similar across the three weight groups, as predicted. Secondary analyses of baseline BNP values across BMI-derived weight groups were unable to demonstrate a natriuretic peptide handicap, as found in previous reports (8, 119). BMI as a continuous variable was also not related to baseline BNP levels. As predicted, there was insufficient power in the present study to address this issue across weight groups.

#### Reactivity of BNP to Mental Stress

HR increased similarly to the cognitive/emotional and mirror tasks, although CO increased with the cognitive/emotional tasks and decreased with the mirror task. Therefore, it follows that the differences in CO reactivity would have to be the result of stroke volume (SV) changes, given that  $CO = HR \times SV$ . Additional analyses showed that SV increased significantly to the cognitive/emotional tasks and decreased significantly to the mirror task. It was

hypothesized that the mirror task would produce a greater increase in BNP than the cognitive/emotional tasks, given the differences in TPR response to these two stressors. Despite the hypothesized pathway, it appears that the difference in SV reactivity, and subsequently CO, was just as crucial as TPR reactivity in BNP responses to the mental stress tasks.

Physiologic Mechanisms of BNP Increases. Given the differences in hemodynamic reactivity, the physiological mechanisms that led to an increase in BNP during mental stress in this study appear to be different for the cognitive/emotional and psychomotor types of mental stress. For the mirror trace task, an increase in afterload most likely produced the increased wall tension that led to BNP release. Afterload has been described by Norton as “all of the factors that contribute to total myocardial wall stress (or tension) during systolic ejection” (3). The Law of LaPlace states that wall stress is equal to ventricular pressure times ventricular radius divided by wall thickness. Circumstances that increase left ventricular output impedance (i.e., hypertension, increased TPR, aortic stenosis, etc.) will necessitate greater systolic ventricular pressure (3).

Afterload is increased in response to an increase in arterial pressure and TPR, as seen in Figure 4 (149), both of which were found in response to the mirror task in this study. Applying the Law of LaPlace to the mirror task in this study, we can assume that wall thickness remained constant, whereas ventricular pressure increased via changes in TPR. Therefore, the increase in afterload led to greater wall stress and BNP release. A decrease in SV was also seen for the mirror task, which can result from increased afterload. It is unclear if

there was an effect of preload during the mirror task because SV was reduced. It is possible that even if there was an increase in preload with the mirror task, it may have been secondary to a stronger increase in afterload.

The increase in BNP found for the cognitive/emotional tasks might reflect a more complex physiological pathway involving the effects of sympathetic nervous system activation and catecholamines. Sympathetic arousal is indicated from previous research showing that catecholamines (i.e., epinephrine and norepinephrine) are increased in response to cognitive types of mental stressors, such as a mental arithmetic challenge (87, 150-152). Infusion of epinephrine results in increased HR, SBP, CO, and SV as well as a decrease in systemic vascular resistance (153). This hemodynamic pattern was found for the cognitive/emotional tasks in the present study. The fact that this pattern was not seen for the mirror task in this study may be related to evidence that the sympathoadrenomedullary system and catecholamine response appears to be more active during cognitive/emotional versus mirror stressors (154).

The effects of catecholamines can create a combination of increased preload and increased contractility (inotropy), both of which increase SV and CO (Figure 16). Specifically, circulating catecholamines decrease venous compliance and increase central venous pressure, which can increase preload. The Frank-Starling mechanism, generated by the increase in preload, allows the heart to eject the additional venous return through greater contraction force (155). If afterload is held constant, the increase in preload results in increased SV in order to handle the excess venous return (156). Additionally, ventricular contractility

(inotropy) can change the contraction force at a given preload and afterload condition (13). Lastly, there is evidence that enhanced sympathetic tone, as seen with mental stressors, can increase BNP directly without a concomitant increase in wall stress (157). Catecholamines are released in response to sympathetic activation and have been shown to induce BNP increases in rats (158). In this study, the infusion of catecholamines from mental stress activation in the cognitive and emotional tasks may have led to increased BNP both directly and indirectly, via increased inotropy and preload (Figure 16).

The results of this study suggest that the mirror task had more of an effect on afterload whereas the cognitive/emotional tasks had more of an effect sympathetic activation and perhaps preload. Although working through different physiological mechanisms, both mental stress tasks were able to bring about similar increases in BNP.

### Sex Differences in BNP

The present study did not find a significant effect of age on BNP that has been found by other investigators (142-144), although the age range was limited in this study as compared to the previous literature. Other investigators have examined ranges from ~20-80 years of age whereas the age range for the present analyses was 20-40 years. One other study did find that although N-ANP and N-BNP varied with age, BNP did not (144). The age range for that study was also narrow as compared to the literature supporting the association of age and BNP levels. Consistent with previous reports, females were found to have

significantly higher BNP than males at all time points in this study. Additionally, for females BNP increased significantly more to the cognitive/emotional tasks whereas for males BNP increased significantly more to the mirror task. This relationship of time, task, and sex may explain why no differences in BNP response were found between the mental stress tasks as predicted in the hypotheses.

One theory explaining why females have been shown to have higher levels of BNP focuses on the role of estrogen and other sex hormones. Female sex hormones have been shown to promote natriuretic peptide gene expression (159) and hormone replacement therapy (HRT) has been associated with higher BNP levels (143). In addition, lower plasma renin levels have been found for females versus males and HRT is associated with even lower renin levels (160). This finding is consistent with the fact that BNP is renin-inhibiting and an antagonist for the renin-angiotensin-aldosterone system (23). Therefore, the evidence is in support of sex hormones as a crucial factor in explaining the significant sex difference in BNP.

#### Hemodynamic Stress Responses in Relation to Obesity

One previous study (106) also found an inverse relationship of BMI with CO and TPR to a cognitive task seen in the present study. However, those investigators did find the expected correlations of TPR and CO with WHR, which was similar to the BMI-based weight group relationships described in other studies (7, 113, 115). It should be noted that the arterial catheter method was

used to obtain CO and TPR during that study (106), which is considered the “gold standard” measurement. Other studies that have found BMI-based weight group relationships with CO and TPR reactivity have utilized the impedance cardiography method. Given this mixed literature, exploratory analyses with body composition measures other than BMI or BMI-derived weight groups were conducted. The present study also found significant correlations of WHR with TPR and CO that were similar to the patterns seen in weight group comparison studies. These findings in the present study were obtained using impedance cardiography, but reproduce the results from a study using arterial catheterization.

Sex differences in WHR ranges could affect these results, so the data were analyzed separately for males and females. There were sex-specific relationships, such that females showed positive correlations of WHR with TPR for both tasks, whereas males showed a positive correlation of WHR with TPR for the mirror task, but a negative correlation of WHR with TPR for the cognitive/emotional tasks. Additionally, the WHR to CO response correlations were no longer significant for either sex. This finding of opposite associations for males and females appears to explain why the overall correlation of WHR and TPR response to the cognitive/emotional tasks was not significant.

WHR vs. BMI. Measures of abdominal adiposity, such as WHR, provide additional or even greater health risk information as compared to overall obesity or BMI (161, 162). In addition, greater cardiovascular stress reactivity has been suggested for individuals with greater central adiposity. One study found greater

TPR and DBP increase to a cognitive stressor in women with central adiposity and greater CO increase in women with peripheral adiposity (i.e., lower WHR) (163). In that same study, BMI was not found to be associated with cardiovascular responses to the stressors (163), suggesting that the predictive value of central adiposity and measures such as WHR are greater than that of BMI. Evidence from investigations of WHR and cardiovascular reactivity to mental stress implicates exaggerated vasoconstriction as the physiological mechanism to explain the difference in reactivity patterns with various measures of obesity and adiposity. The positive correlation of WHR and TPR response to both stressors for women in our study also indicates a pattern of vasoconstriction in response to various types of psychological stress with greater abdominal adiposity, but not greater BMI. Repeated stress reactions such as these may play a role in the greater cardiovascular disease risk associated with increased WHR. The results from this study corroborate that WHR may be a greater predictor of unhealthy cardiovascular reactivity patterns and cardiovascular disease risk as compared with BMI. However, these particular relationships may be most prominent among women.

Adipose tissue distribution is a possible explanation of sex differences found for how WHR related to CO and TPR reactivity. Visceral adipose tissue (AT) has been shown to have greater negative consequences as compared to subcutaneous AT (164). Visceral fat accumulation has been implicated in the development of cardiovascular disease and hypertension (165) and is accompanied by elevated blood pressure and negative cholesterol profiles (166).

Men have been shown to have greater amounts of visceral AT and less subcutaneous AT compared with females (167-169). Given the sex differences in visceral versus subcutaneous AT and the negative health effects of visceral AT, it would follow that the relationship of WHR to cardiovascular reactivity should be more unfavorable in men. However, in the present study the association of WHR with reactivity was worse for women. It is possible that the mere fact that women have been shown to have greater adiposity as compared to men (170) may be sufficient to produce sex differences in reactivity patterns as opposed to specific types of adipose tissue. Or perhaps females in the present study, who were more represented in the obese group, in fact had greater visceral AT than males. The measure of WHR is not able to distinguish between visceral and subcutaneous AT in the abdomen, so the role of adipose tissue distribution remains unclear.

#### Reactivity of BNP in Relation to Obesity

For this study, the research model purports that the natriuretic peptide handicap found for obese individuals at rest is not a consequence of BNP production/secretion dysfunction, but rather due to adipose tissue receptor concentration increasing BNP clearance. Therefore, the finding that the weight groups were able to demonstrate similar BNP increases to mental stressors provides evidence that production of BNP may not bring about the natriuretic handicap.

Further evidence supporting the adipose tissue clearance hypothesis was found when WHR was compared to BNP at rest and in response to mental stress

in this study. Because of the sex differences in both BNP and WHR, the focus will remain on the sex-specific results. In particular, for females, higher WHR was associated with lower baseline levels of BNP, supporting a BNP handicap at rest. However, no association of WHR and baseline BNP was found for males. For females, higher WHR was also associated with greater increase in BNP to the mirror trace task, which approached significance. Regardless of the significance of that result, both males and females did not show a reduced BNP response with higher WHRs, which does not support the dysfunctional BNP production theory.

It was unexpected to find evidence of a BNP handicap with the WHR measure, at least in females, as opposed to BMI or weight groups. However, this result now appears fitting given that WHR is mainly determined by adipose tissue whereas BMI may not (106). It follows that those with higher WHRs would have a greater number of natriuretic peptide receptors due to their abundance in adipose tissue and could clear greater amounts of BNP at rest as compared to those with lower WHRs. It is possible that in previous literature on BNP handicap with BMI-derived weight groups (8), WHR would have shown a similar BNP pattern. However, those analyses were not conducted, so it is unclear if BMI or WHR account for greater amounts of the variance in resting BNP, which could provide information on the accuracy of the adiposity theory. Because the present study was underpowered to address the BNP handicap issue with BMI, the WHR association found here in females needs to be replicated with a larger sample powered to investigate various measures of body composition.

### Study Limitations

Statistical Power. Although many of the hypotheses of this study were confirmed, there were limitations that qualified the information gained from this project. The small sample size (N=33) was an important limitation. Adequate power was obtained for most of the analyses in Hypothesis 1, whereas many of the weight group specific analyses in Hypothesis 2 were underpowered (see Table 3). In particular, analyses that required separate analyses for males and females either due to BNP or WHR sex differences may have been limited by the small sample sizes in each cell. In particular, sex differences found in CO/TPR patterns and BNP to WHR relationships may be due to limited power in the male group (N=10 versus N=23). Additionally, the overweight group consisted of only 5 individuals, which reduced the power of weight group analyses. It should be noted that collapsing the overweight and obese groups did not resolve this issue, mainly because the pattern of results was generally similar between the obese and normal groups, but not the overweight group.

In addition, future studies would benefit from having increased sample size in order to evaluate the role of race on hemodynamic and BNP reactivity to mental stress and exercise. Previous literature has shown that African-Americans exhibit stronger peripheral vascular responses to a cold pressor challenge (171) and there are racial differences in hemodynamic reactivity to mental stressors (172, 173). The data collected for the present study had cell sizes that were too small to detect any meaningful differences, especially for measures that needed

to be controlled for by sex. It would also be useful to determine if any of the previous findings regarding obesity and cardiovascular reactivity to stress could be moderated by race.

Sample Limitations. Another study limitation was the fact that only normal and overweight military members, beneficiaries, and federal employees were allowed to participate in the exercise testing. The effectiveness of the mental stressors may have been different between those in the military and those who are not. For example, it is possible that military members, who may be used to high levels of stress, are not challenged by the tasks they were asked to perform. It is also possible that for those in the military, the anticipation of the task is more stressful than the task itself. When they perform the task it is not as stressful as they expected and may therefore decrease their responses as compared to resting values. It would be useful to design a study that could better address the saliency and effectiveness of these types of stressors in military and civilian samples.

Even though the sample size of participants who underwent exercise testing was small, the effect size for BNP response to exercise was strong enough to show significance. However, it would have been useful to conduct exercise testing in all three weight groups with adequate sample size to further address the BNP production versus reuptake hypotheses of natriuretic handicap. The much larger BNP response found for exercise as compared to mental stress may enhance the ability to detect weight group differences in BNP response to challenge. In addition, assessing level of fitness on all participants would have

provided valuable information because there is evidence that increased aerobic fitness is associated with reduced cardiovascular reactivity to mental stress (174, 175). This relationship could help explain the differences in mental stress reactivity across WHR and BMI found in the present study and previous literature.

Lack of Other Blood Markers. Lastly, no other blood measures (i.e., endocrine markers, other natriuretic peptides, hematocrit, etc.) were assessed for comparison with BNP and hemodynamic responses. Measurement of other natriuretic peptides (i.e., ANP and NT-proBNP) could provide a clearer representation of how different types of challenges stimulate the natriuretic peptide system and the consequences of each peptide's release. However, the choice of BNP for this study was made purposefully because it was believed to be the most informative and appropriate for the type of testing included in the study. BNP has a 2-3 times more powerful effect on natriuresis and BP lowering than ANP (176) and BNP has a longer half-life and more stable release pattern (177). In addition, measurement of catecholamines would have allowed exploration of mechanisms involved in mental stress-induced hemodynamic and BNP reactivity.

Another reason why it would have been beneficial to assess other blood measures involves the issue of hemoconcentration, which is an increase in the concentration of cellular and other components of the blood generally due to a reduction in plasma volume (178). Biochemical measures, including hormones, may be affected by transcapillary movement of water (97). There is a possibility

that increases in BNP in response to the tasks conducted in the present study could be the result of hemoconcentration that has been shown to occur with mental stress (178-180) and exercise (97) and may be mediated by catecholamines that result in decreased plasma volume (181). A decrease in plasma volume in response to the mental and physical stressors could falsely indicate an increase in BNP, when in fact BNP remained relatively constant, but appeared increased due to a change in the ratio of BNP to the total sample. However, there is also some evidence that atrial natriuretic peptide (ANP), and perhaps natriuretic peptides in general, reduce plasma volume as part of their compensatory actions and to decrease volume overload (182, 183). Research in dogs showed that an infusion of BNP increased hematocrit, which could be a result of hemoconcentration (184). In addition, one study that controlled for hematocrit still found a significant increase in BNP in response to exercise (97). Therefore, it appears unclear if hemoconcentration effects result in the appearance of BNP increases or if BNP increases result in hemoconcentration.

The current study was setup to address the connection between a limited set of variables in response to stressors in order to determine if further investigation into mechanisms was warranted. Future research should investigate a broader set of biological markers in order to better understand the physiological pathways involved in mental and physical stressors.

### Study Implications

An implication from this study is the notion that WHR may be a better predictor of stress reactivity and disease risk as compared with BMI. There is already evidence that WHR is associated with increased incidence of ischemic heart disease, stroke, and death (185), but the comparison of WHR with BMI has found WHR to show increased disease risk as compared with BMI. A study of women found that WHR was associated with most of the traditional cardiovascular risk factors, but this association was not found for BMI (186). In a multinational study, WHR was shown to be a better predictor of myocardial infarction than BMI (187). The Heart Outcomes Prevention Evaluation (HOPE) study found that individuals with high waist circumference measurements had greater risk of heart failure, myocardial infarction, and total mortality (100). Also, women in that study showed an increased risk of heart failure with increasing WHR (100). The results from the present study are consistent with these previous investigations, such that WHR was associated with poorer hemodynamic and BNP reactivity as compared with BMI, especially in women. In addition, WHR was related to a natriuretic peptide handicap (lower baseline BNP) in this study. This handicap may help explain the link between WHR and cardiovascular disease. Specifically, lower levels of circulating BNP at rest may result in reduced ability of the cardiovascular system to compensate to a stressor in individuals with high WHR.

Measures of abdominal adipose tissue provide greater value for risk prediction across ethnic groups (188-191) and at older ages as compared with BMI (192-194). For these reasons, as well as the evidence from the present

study, it appears that using WHR, in addition to BMI, could greatly increase cardiovascular disease and heart failure risk prediction across a diverse population.

BNP Increases with Mental Stress. To our knowledge, this study is unique in demonstrating that mental stressors could produce significant BNP increases. Moreover, no other researchers have addressed mental stress reactivity of BNP in young, healthy, disease-free individuals.

To more fully understand the effects of mental stress on BNP, future research could focus on various types of stress (i.e., chronic, severe acute, traumatic, perceived) to determine which ones produce the greatest effects on the natriuretic peptide system. If groups differing on BNP responses can be identified (i.e., WHR categories), then BNP reactivity may be useful in risk stratification. Higher resting natriuretic peptide levels in non-diseased individuals have been shown to predict risk of death and cardiovascular events during follow-up even after adjusting for traditional risk factors (29). The risk from BNP was strongest for incident heart failure, which corresponds physiologically given that higher levels of BNP suggest greater need for homeostasis in the cardiovascular system. For those individuals, the imbalance/overwork of the cardiovascular system could eventually result in heart failure.

One possible reason for elevated BNP and incident heart failure could result from chronic or repeated experience of psychological stress, which we now know can increase BNP due to the need of the cardiovascular system to return to homeostasis. Excess amounts of preload and afterload resulting from daily life

stress could result in left ventricular remodeling and dysfunction. Although the percent change of BNP to the mental stress tasks was relatively small (3-21%), there was variability in response to mental stress. It is possible that those who showed the strongest response to the laboratory mental stressors in this study find the stressors more salient or are more prone to a physiological response to stress. Therefore, BNP reactivity may be useful for identifying healthy individuals who are more cardiovascularly affected by mental stress challenges, such as those experienced regularly in daily life. In addition, future research addressing BNP reactivity to mental stress in heart failure patients would provide another layer of information. If BNP in heart failure patients increases 50% or more in response to stressors, there would be evidence that stress reactivity of the cardiovascular and natriuretic peptide systems could be a clinically relevant indicator of decompensation (23). Furthermore, reduction in stress levels of heart failure patients, through stress management techniques, could reduce hospitalizations and increase quality of life.

Mental stress-induced increases in BNP seen in this study appear to reflect changes in preload and afterload, which could indicate greater heart failure risk. Psychological stress, along with hypertension, smoking, and high WHR, has been shown to be a risk factor for heart failure (64). There are various definitions and types of psychological stress, which can sometimes be difficult to quantify. Having an objective marker like BNP reactivity could provide an easier way to quantify excess risk of future disease and events associated with stress. For example, exercise-induced BNP response can increase the sensitivity for

detecting ischemia (195). Therefore, mental stress-induced BNP response may also provide increased sensitivity for disease detection and prediction, especially for heart failure, in healthy individuals and those with suspected heart disease.

### Summary

In sum, this study yielded three key findings: (1) Mental stress tasks were effective in producing hemodynamic reactivity, which replicated previous patterns comparing cognitive/emotional (math/speech) versus psychomotor (mirror trace) stressors; (2) Mental stress tasks increased BNP, although the increase produced by exercise was greater; (3) Lastly, WHR was found to be a better indicator of hemodynamic and BNP reactivity than BMI or weight groups derived from BMI, particularly in females. Mental stress reactivity of BNP and WHR may therefore be useful for cardiovascular disease risk prediction, even in young, healthy, disease-free individuals.

*Table 1.* New York Heart Association Classification of Heart Failure (1)

Class I	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.
Class II	Slight limitation of physical activity. Comfortable at rest. Moderate physical activity results in fatigue, palpitation, dyspnea, or anginal pain.
Class III	Marked limitation of physical activity. Comfortable at rest. Minimal activity causes fatigue, palpitation, dyspnea, or anginal pain.
Class IV	Inability to carry on any physical activity without discomfort. Symptoms of heart failure may be present at rest. Discomfort increased with any physical activity.

Table 2. Sample characteristics by weight groups

	Normal Weight	Overweight	Obese
N	18	5	10
Age	27.67 ± 4.97	32.80 ± 4.49	31.10 ± 6.15
Female (%)	14 (78)	1 (20) *	8 (80)
Caucasian (%)	13 (72)	3 (60)	4 (40)
African American (%)	2 (11)	0	5 (50) *
Years of Education	17.41 ± 2.76	15.40 ± 8.65	17.40 ± 5.04
Current Smoker (%)	2 (11)	0	2 (20)
Body Mass Index (kg/m <sup>2</sup> )	23.33 ± 1.17	27.17 ± 1.13 *	33.68 ± 2.86 * <sup>+</sup>
Baseline SBP (mm Hg)	117.11 ± 7.37	123.40 ± 2.97	125.10 ± 7.37 *
Baseline DBP (mm Hg)	69.78 ± 5.62	74.40 ± 5.86	75.40 ± 6.80 *
Baseline HR (bpm)	67.61 ± 10.18	67.40 ± 5.51	66.40 ± 13.24

\*  $p < 0.05$  compared with Normal Weight group

<sup>+</sup>  $p < 0.05$  compared with Overweight group

*Table 3.* Effect sizes and power calculations observed in this study

<b>Analysis</b>		<b>n</b>	<b>Partial Eta<sup>2</sup></b>	<b>Power (%) <sup>a</sup></b>
SBP Reactivity	C/E	33	0.70	100
	Mirr	33	0.70	100
DBP Reactivity	C/E	33	0.79	100
	Mirr	33	0.68	100
HR Reactivity	C/E	33	0.59	100
	Mirr	33	0.73	100
CO Reactivity	C/E	32	0.43	99.7
	Mirr	32	0.49	100
TPR Reactivity	C/E	32	0.06	25.5
	Mirr	32	0.55	100
BNP Reactivity	C/E	32	0.25	88
	Mirr	32	0.17	67.6
	EX	8	0.97	100
BNP Reactivity x MS Tasks		31	0.02	10.3
BNP Reactivity x All Tasks		8	0.96	100
CO Reactivity x Weight Grps	C/E	32	0.10	30.8
	Mirr	32	0.02	10.1
TPR Reactivity x Weight Grps	C/E	32	0.18	56
	Mirr	32	0.07	21.8
BNP Reactivity x Weight Grps	C/E	32	0.13	39.1
	Mirr	32	0.003	5.6
Baseline BNP x Weight Grps		33	0.06	20.9

<sup>a</sup> All power calculations based on  $\alpha = 0.05$

Abbreviations: SBP = Systolic blood pressure (mm Hg); DBP = Diastolic blood pressure (mm Hg); HR = Heart rate (bpm); CO = Cardiac output (L/min); TPR = Total peripheral resistance ( $\text{dyn}\cdot\text{s}\cdot\text{cm}^{-5}$ ); BNP = B-Type Natriuretic Peptide (pg/mL); C/E = Cognitive/Emotional Tasks; Mirr = Mirror Task; EX = Exercise Task; MS = Mental stress

*Table 4.* Mean responses (stress-rest) of hemodynamic variables to both types of mental stress.

	Cognitive/Emotional			Mirror		
	Mean Change	<i>t</i>	<i>p</i>	Mean Change	<i>t</i>	<i>p</i>
SBP (mm Hg)	12.55	8.68	<0.001	9.68	8.66	<0.001
DBP (mm Hg)	11.69	10.96	<0.001	8.35	8.27	<0.001
HR (bpm)	9.29	6.80	<0.001	8.83	9.18	<0.001
CO (L/min)	1.11	4.87	<0.001	-0.63	-5.44	<0.001
TPR (dyn·s·cm <sup>-5</sup> )	-91.86	-1.34	<i>p</i> =0.19	425.65	6.19	<0.001

Table 5. Results from the 2x2 within-subjects repeated measures ANOVAs for each hemodynamic variable.

Cardiovascular Measures	<i>F</i> -ratios			Time X Task			
	Time	Task	Time X Task	Cognitive/Emotional		Mirror	
				B <sup>a</sup>	T <sup>a</sup>	B	T
HR (bpm)	93.16*	1.19	0.11	62.94	72.23	62.39	71.22
SBP (mm Hg)	116.91*	4.31*	3.35	116.92	129.47	116.68	126.36
DBP (mm Hg)	153.12*	5.01*	6.60*	70.93	82.62	70.89	79.24
CO (L/min)	3.15	45.25*	51.51*	5.07	6.18	5.25	4.62
TPR (dyn·s·cm <sup>-5</sup> )	15.95*	15.20*	22.59*	1564.4	1472.6	1485.2	1910.8

<sup>a</sup> B = Baseline, T = Task

\*  $p < 0.05$

*Table 6.* Mean levels of BNP and log transformed BNP before and after each task.

	<b>Cognitive/Emotional</b>		<b>Mirror</b>		<b>Exercise</b>	
	Pre	Post	Pre	Post	Pre	Post
BNP (pg/mL)	17.81 ± 13.37	19.16 ± 13.63	17.91 ± 13.73	18.78 ± 13.68	16.75 ± 10.63	45.25 ± 27.09
Log BNP	2.26 ± 0.67	2.74 ± 0.65 *	2.65 ± 0.68	2.73 ± 0.63 <sup>+</sup>	2.67 ± 0.58	3.68 ± 0.53 *

\* p < 0.01 compared with pre-task levels

<sup>+</sup> p < 0.05 compared with pre-task levels

Table 7. Cardiac output by weight groups

	Cognitive/Emotional		Mirror	
	Rest	Stress	Rest	Stress
Normal	5.83 ± 1.35	7.09 ± 2.10	6.05 ± 1.57	5.36 ± 1.63
Overweight	4.28 ± 1.12	4.46 ± 1.09	3.91 ± 0.78	3.50 ± 0.86
Obese	4.17 ± 1.47	5.49 ± 2.34	4.56 ± 1.60	3.91 ± 1.56
$F_{(2,29)}^a$	5.72	3.89	5.56	4.45
$p$	<0.01	<0.04	<0.01	<0.03

<sup>a</sup> One-way ANOVAs comparing the three weight groups on levels of cardiac output

Table 8. Total peripheral resistance by weight groups

	Cognitive/Emotional		Mirror	
	Rest	Stress	Rest	Stress
Normal	1207.14 ± 314.87	1171.35 ± 312.07	1177.02 ± 326.73	1515.27 ± 467.92
Overweight	1833.19 ± 678.16	1987.31 ± 809.55	1879.50 ± 471.28	2328.11 ± 763.36
Obese	2037.43 ± 988.16	1727.25 ± 746.13	1811.90 ± 734.06	2374.67 ± 1152.58
$F_{(2,29)}^a$	5.66	5.55	6.74	4.63
$p$	<0.01	<0.01	<0.01	<0.02

<sup>a</sup> One-way ANOVAs comparing the three weight groups on levels of cardiac output

*Table 9.* Pearson correlations of WHR and body fat percentage to rest and stress levels of BNP for both types of mental stress

	Cognitive/Emotional		Mirror	
	Rest	Stress	Rest	Stress
WHR	$r=-0.45, p<0.02$	$r=-0.46, p<0.01$	$r=-0.47, p<0.01$	$r=-0.43, p<0.02$
Body Fat %	$r=0.36, p<0.05$	$r=0.43, p<0.02$	$r=0.36, p<0.05$	$r=0.40, p<0.03$

Figure 1. Heart Failure Process (2)

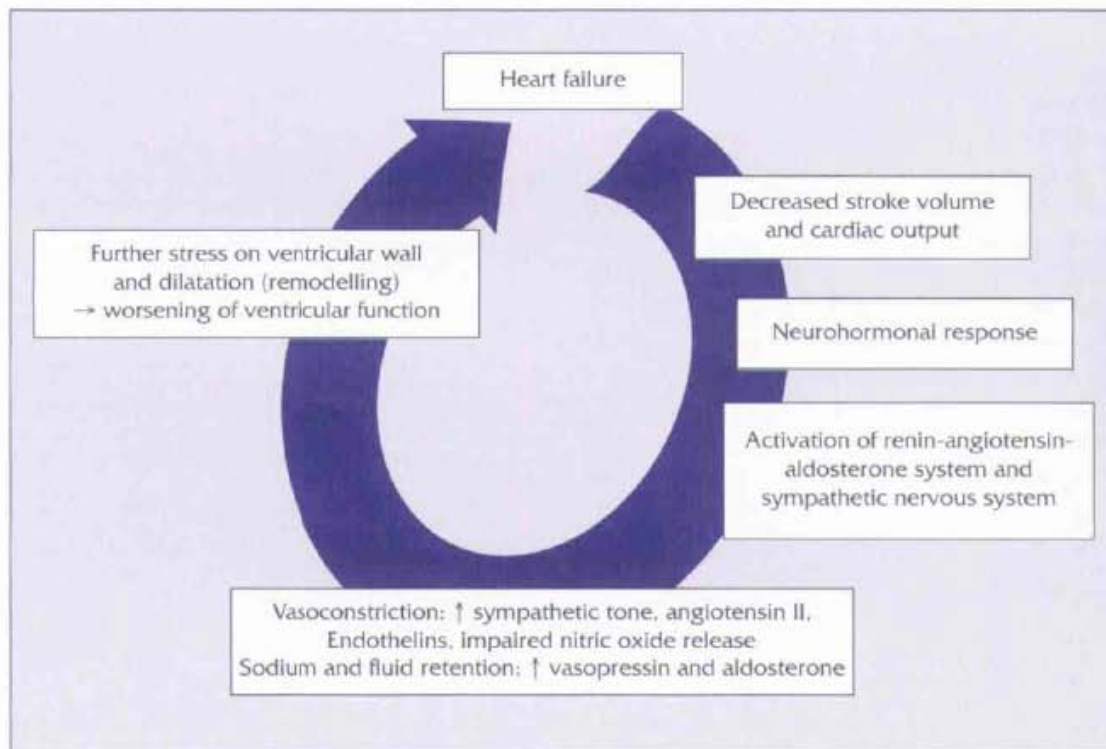


Figure 2. Research Model

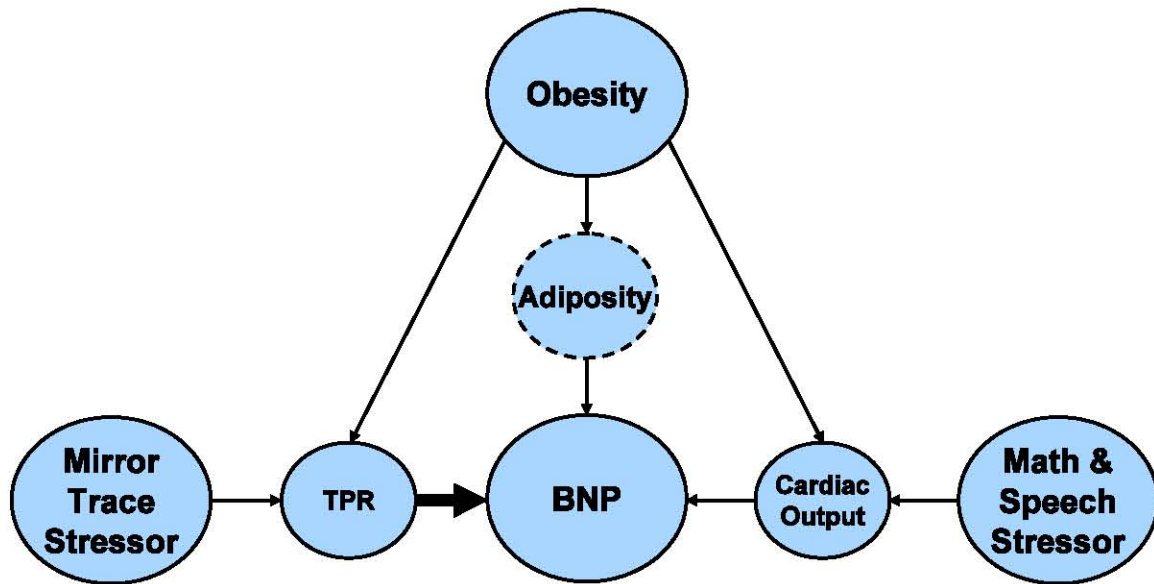


Figure 3. Preload Diagram Indicating that Total Peripheral Resistance is a Key Factor (3)

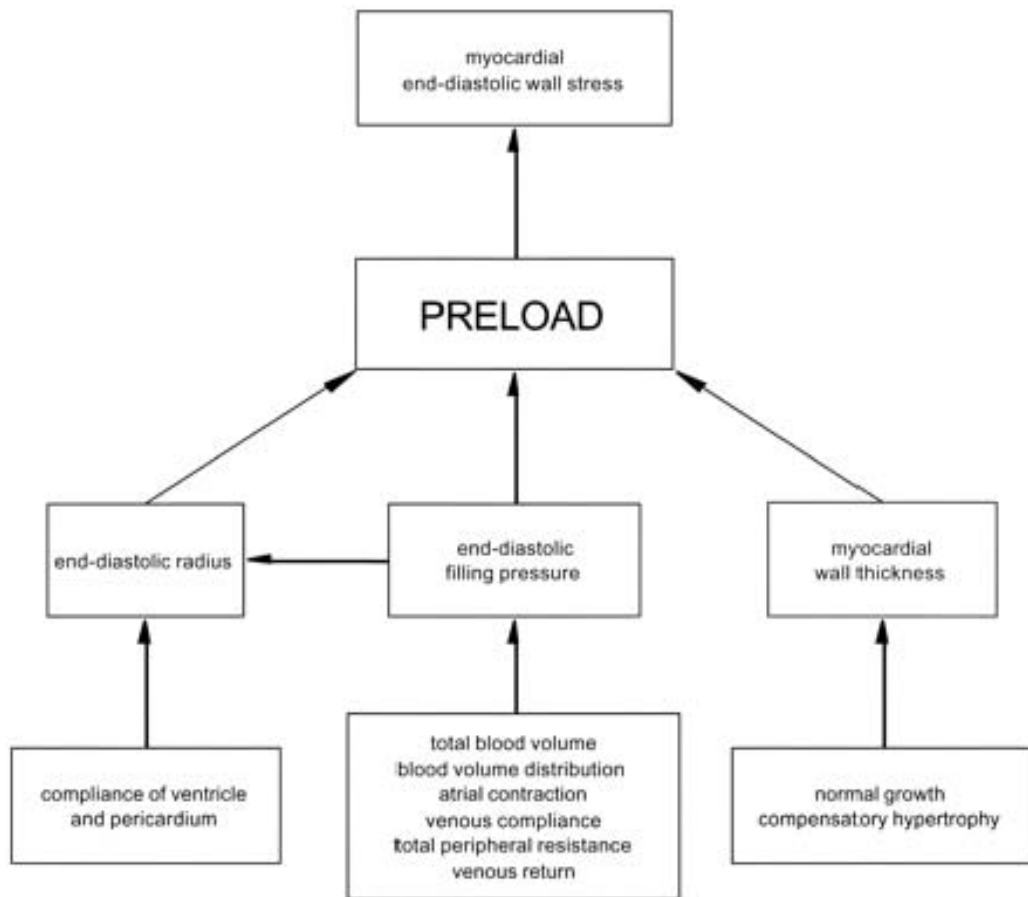
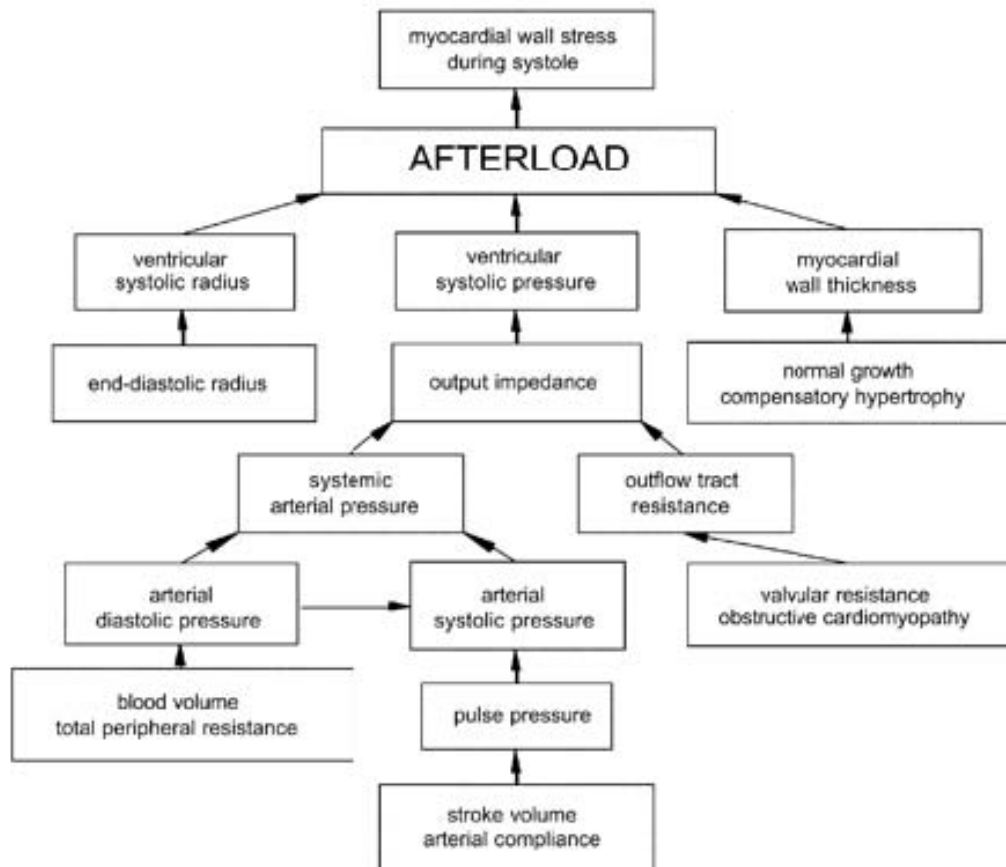


Figure 4. Afterload Diagram Indicating that Total Peripheral Resistance is a Key Factor (3)



*Figure 5.* Timeline of Study Procedures for Mental Stress Only (Top) and Mental Stress Plus Exercise (Bottom)

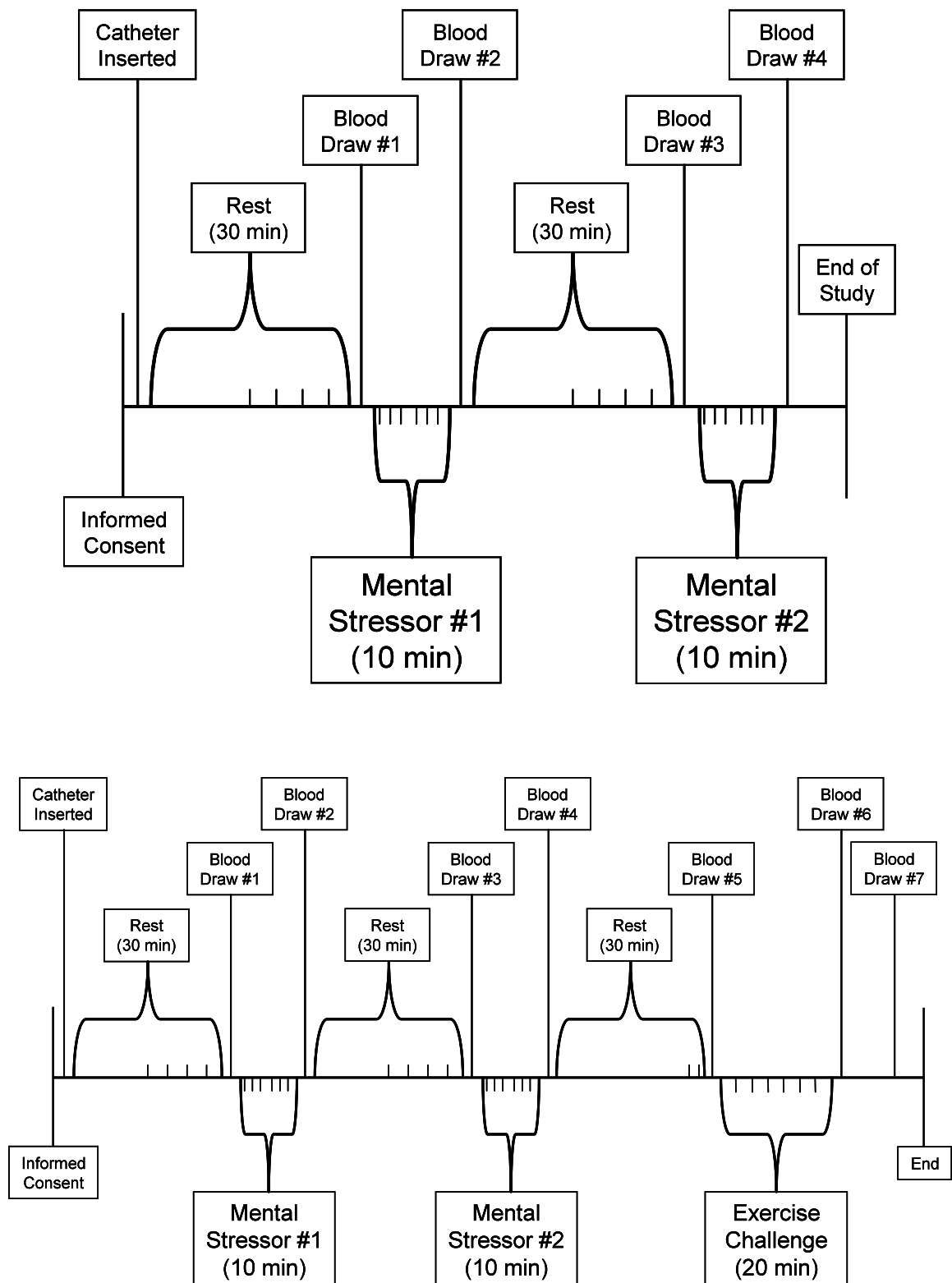
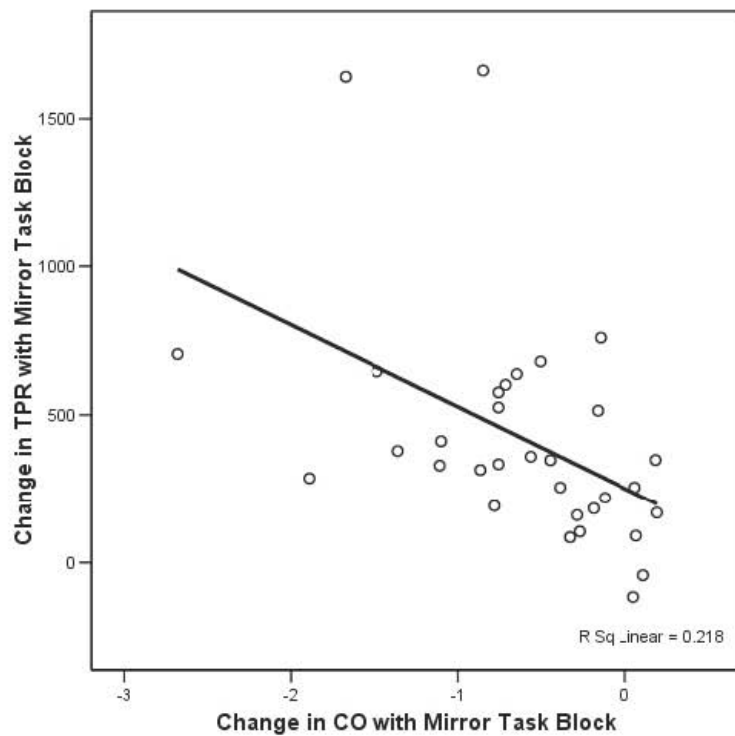
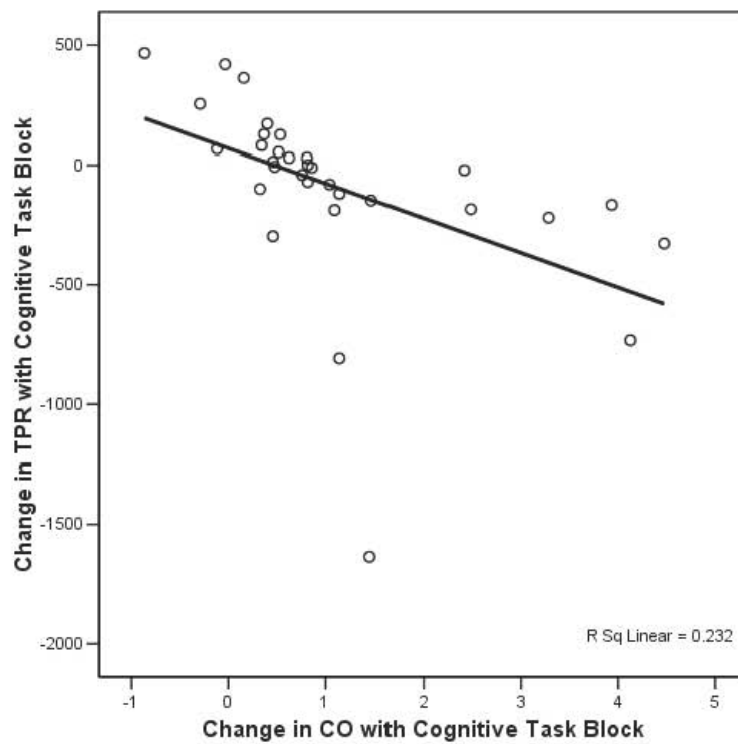


Figure 6. Relationship of CO (L/min) Changes with TPR (dyn·s·cm<sup>-5</sup>) Changes to the Cognitive/Emotional (Top) and Mirror (Bottom) Tasks



*Figure 7. Cardiac Output (L/min) Response to the Cognitive/Emotional Tasks (Solid Line) and Mirror Task (Dotted Line)*

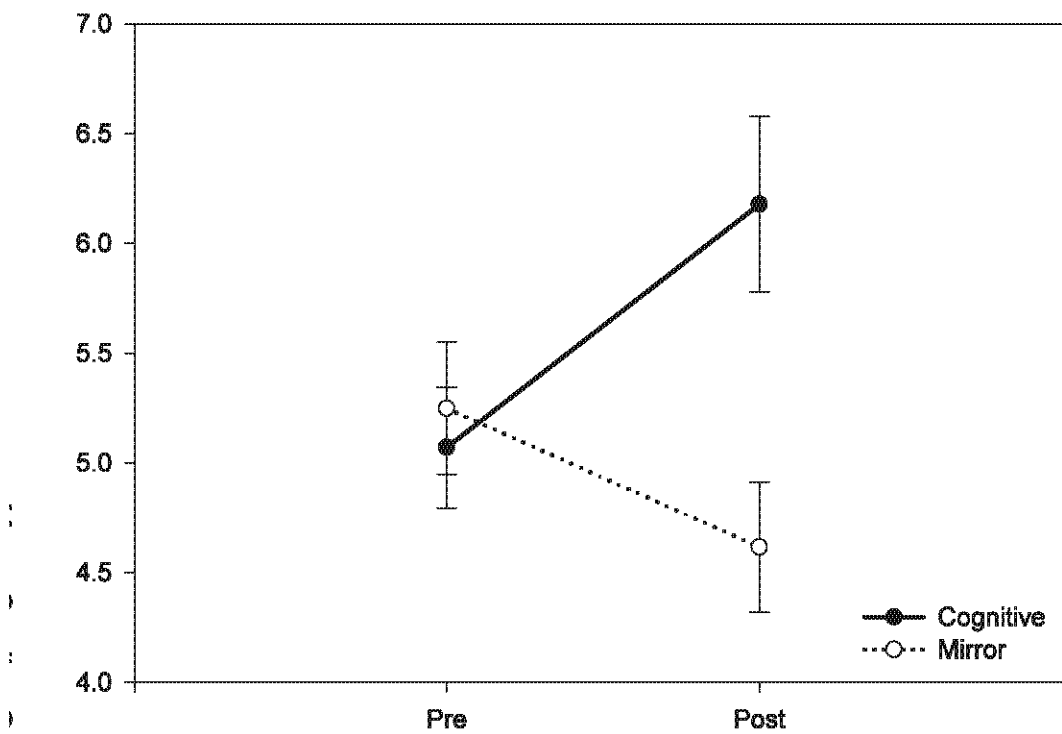


Figure 8. Total Peripheral Resistance ( $\text{dyn}\cdot\text{s}\cdot\text{cm}^{-5}$ ) Response to the Cognitive/Emotional Tasks (Solid Line) and Mirror Task (Dotted Line)

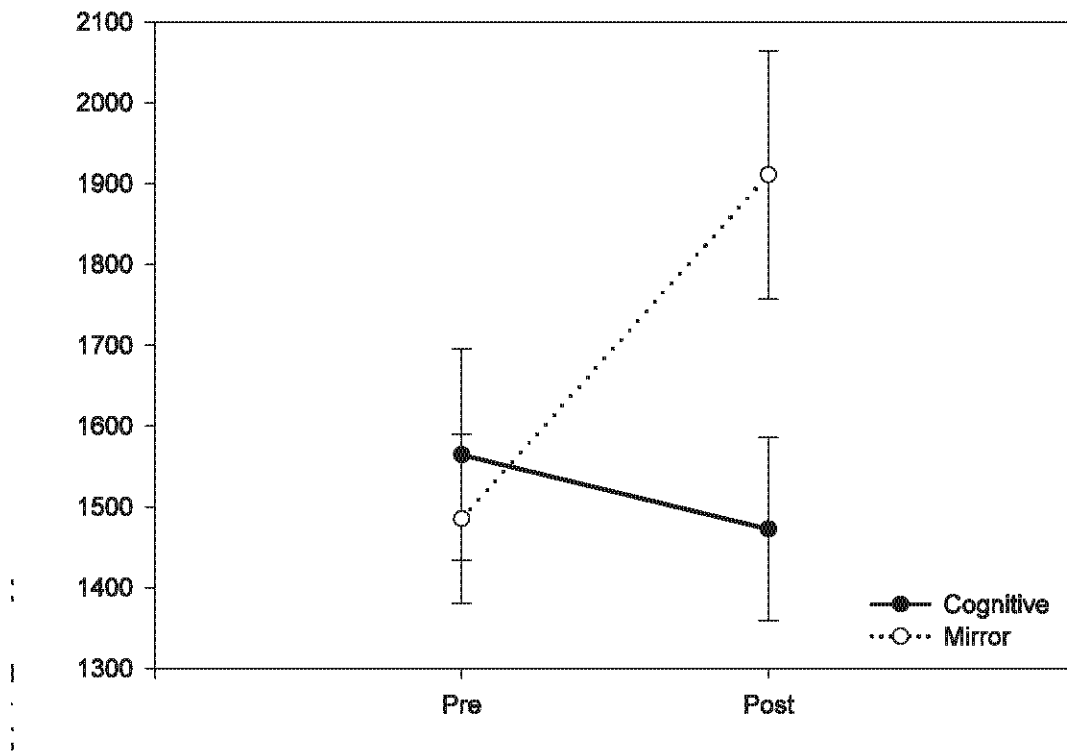


Figure 9. Distribution of Raw BNP Levels Before and After Mental Stressors

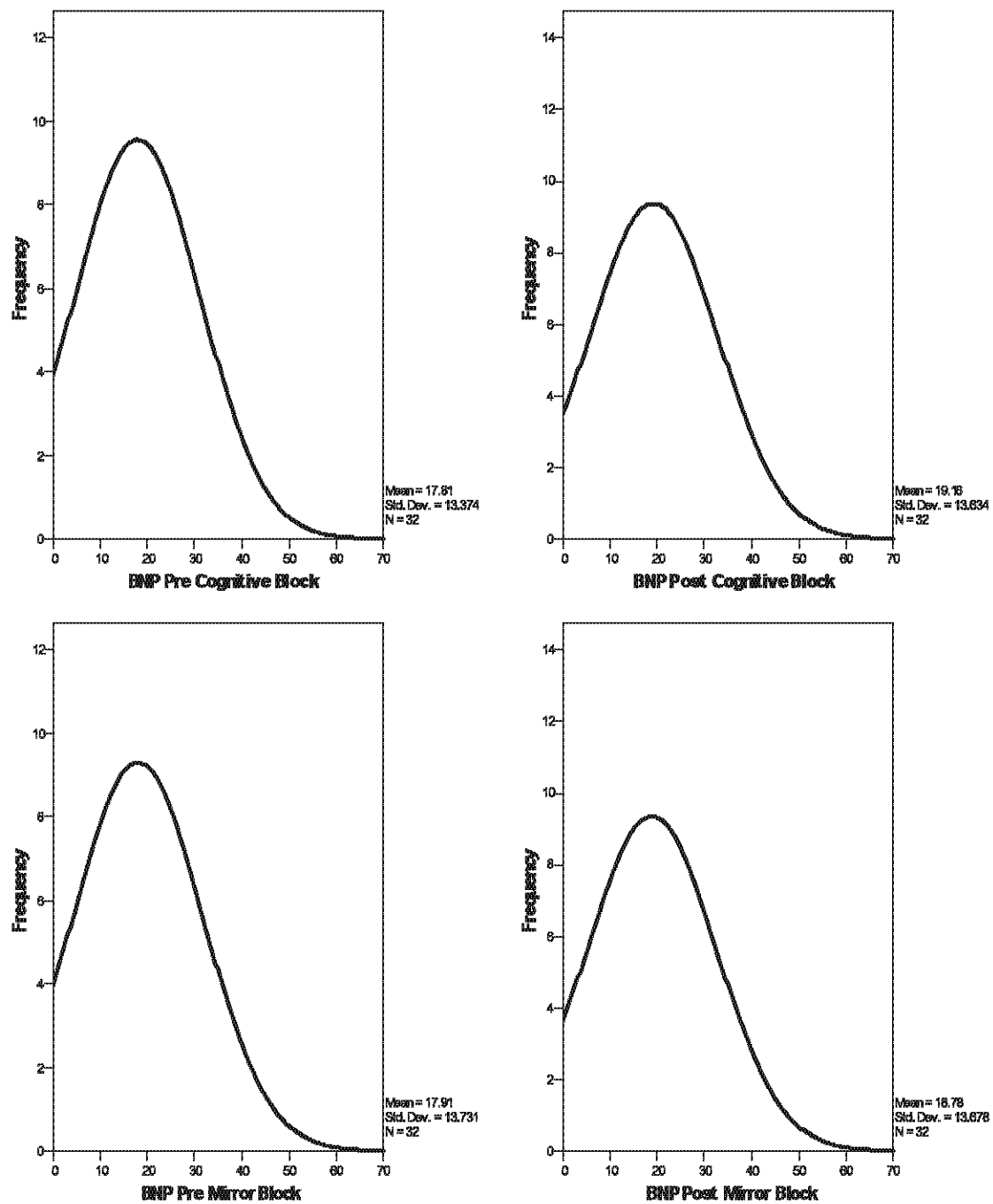


Figure 10. Log-transformed BNP (pg/mL) Response to Cognitive/Emotional Stress (Solid Line), Psychomotor Stress (Dotted Line), and Exercise (Dashed Line)

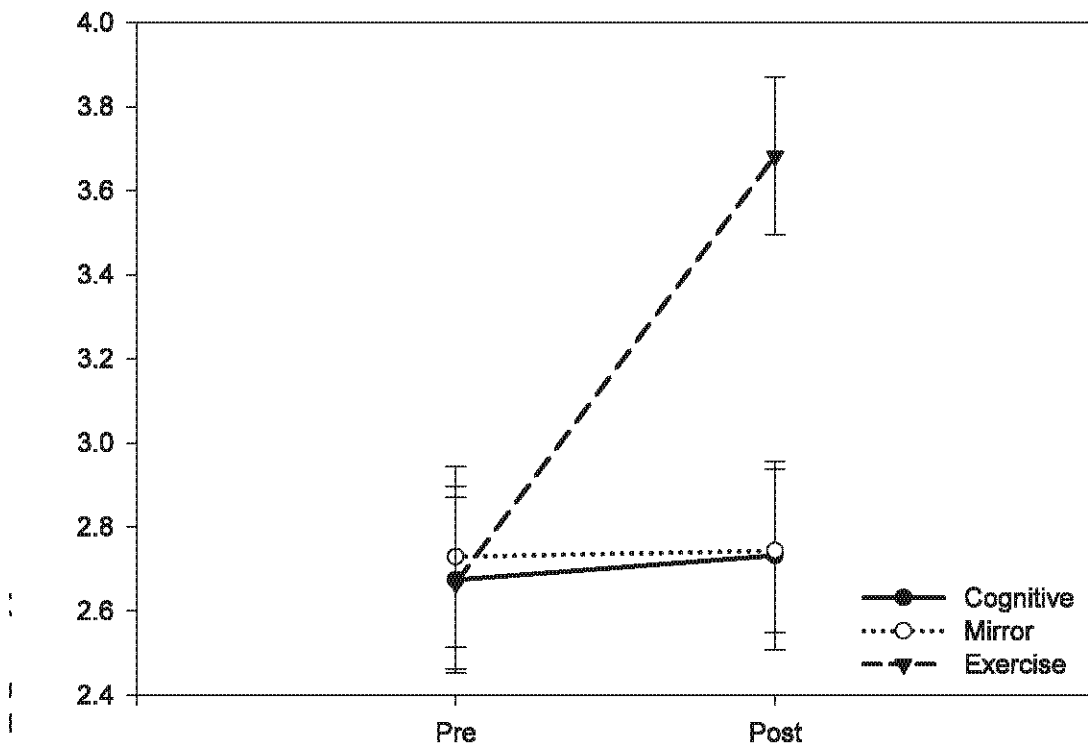


Figure 11. Log-transformed BNP (pg/mL) Response to the Cognitive/Emotional Mental Stress Tasks Comparing Females (Dotted Line) to Males (Solid Line)

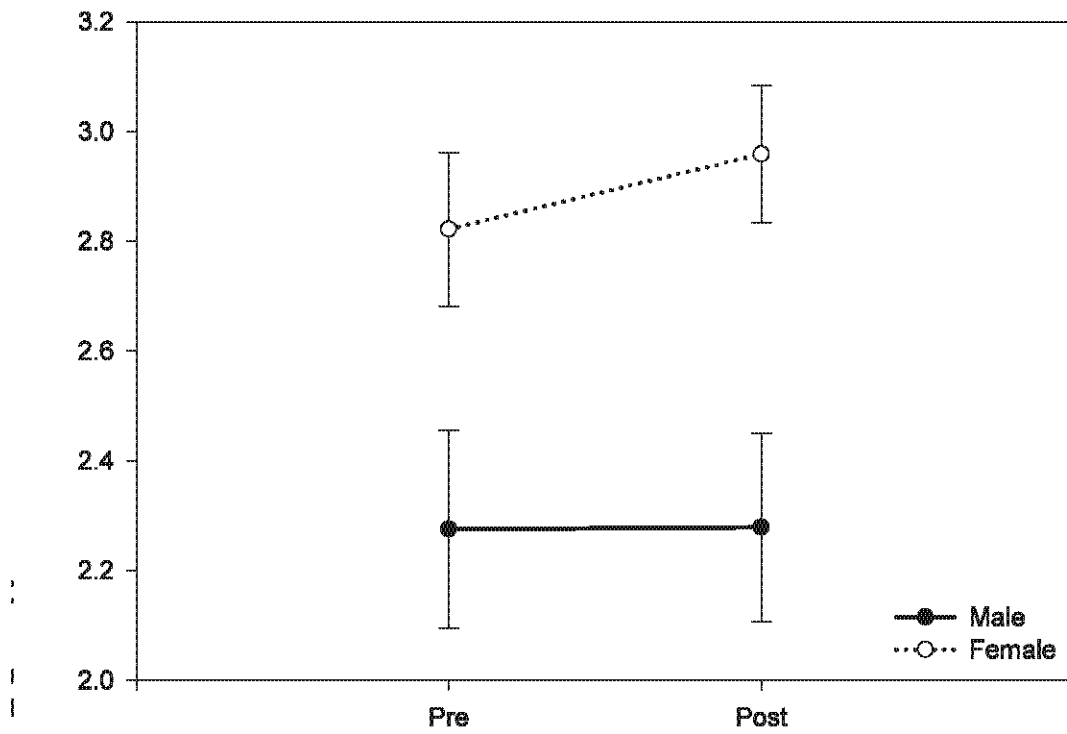


Figure 12. Log-transformed BNP Response to Mental Stressors Presented Separately for Males (Top) and Females (Bottom)

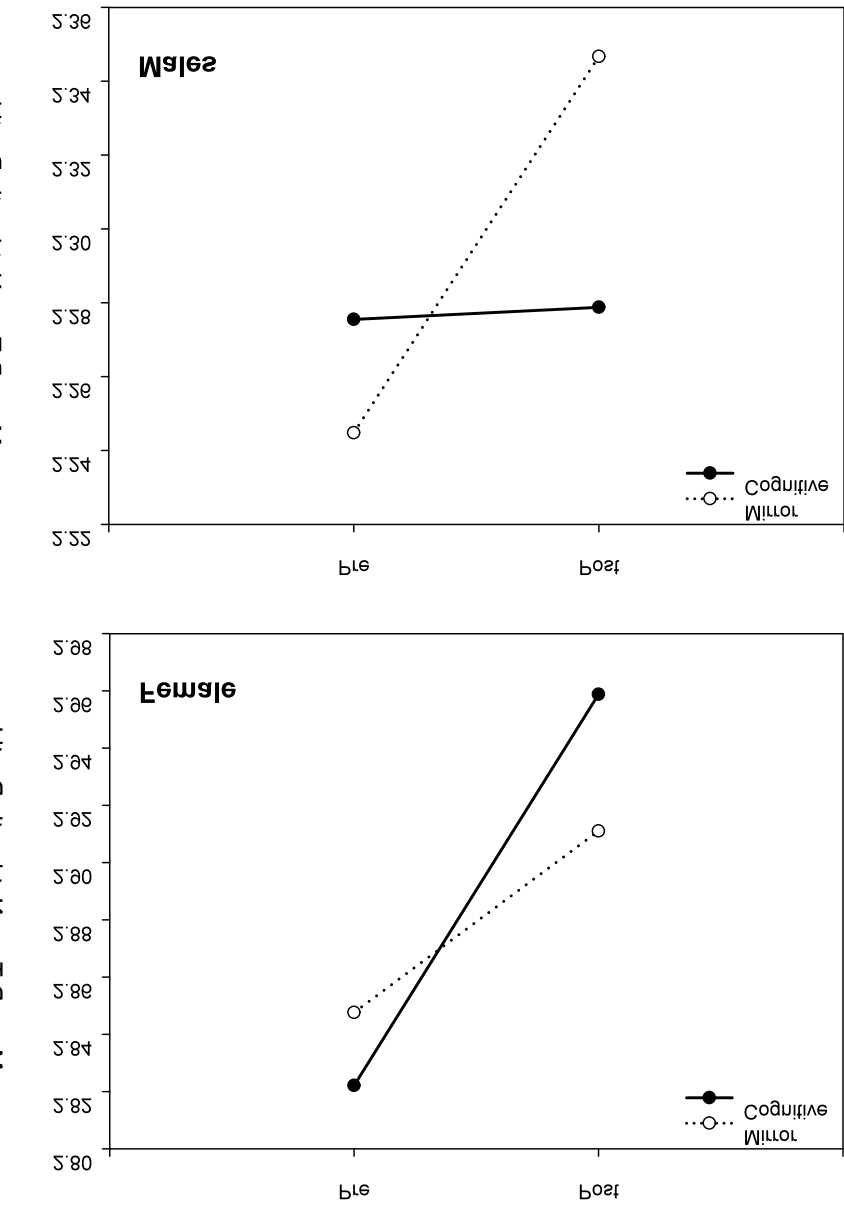
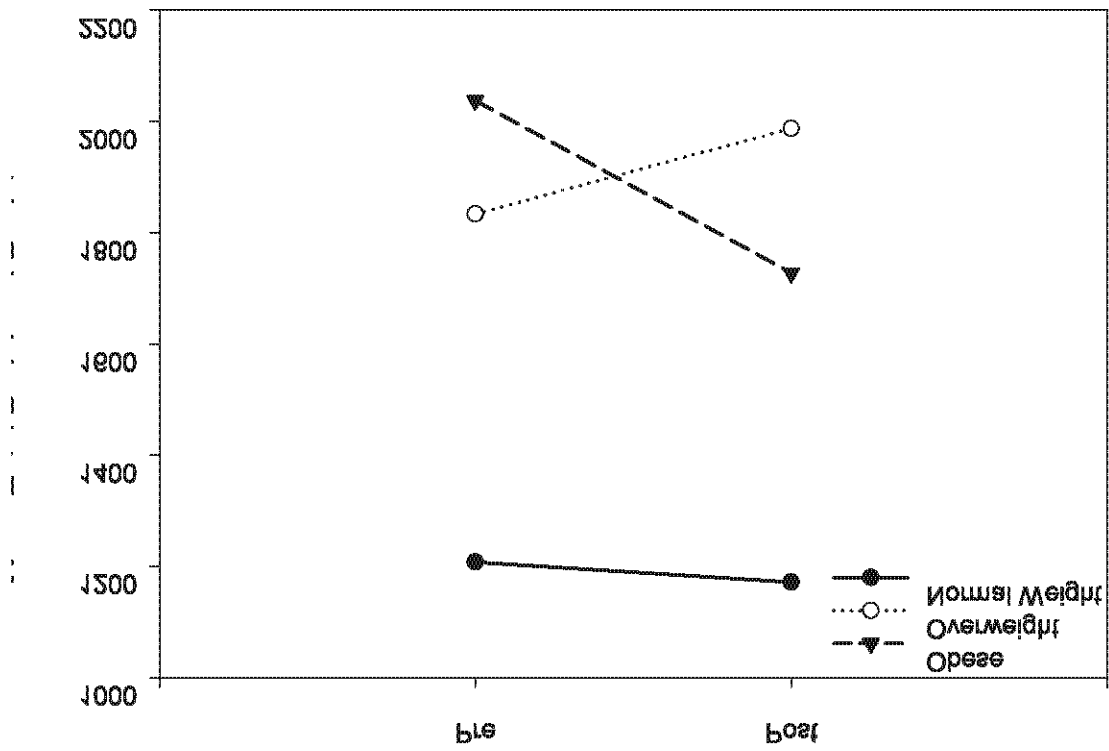


Figure 13. Total Peripheral Resistance ( $\text{dyn}\cdot\text{s}\cdot\text{cm}^{-5}$ ) Response to the Cognitive/Emotional Mental Stress Tasks for the Three Weight Groups



**Figure 14.** Total Peripheral Resistance ( $\text{dyn}\cdot\text{s}\cdot\text{cm}^{-5}$ ) Response to Both Mental Stressors Presented Separately for the Three Weight Groups

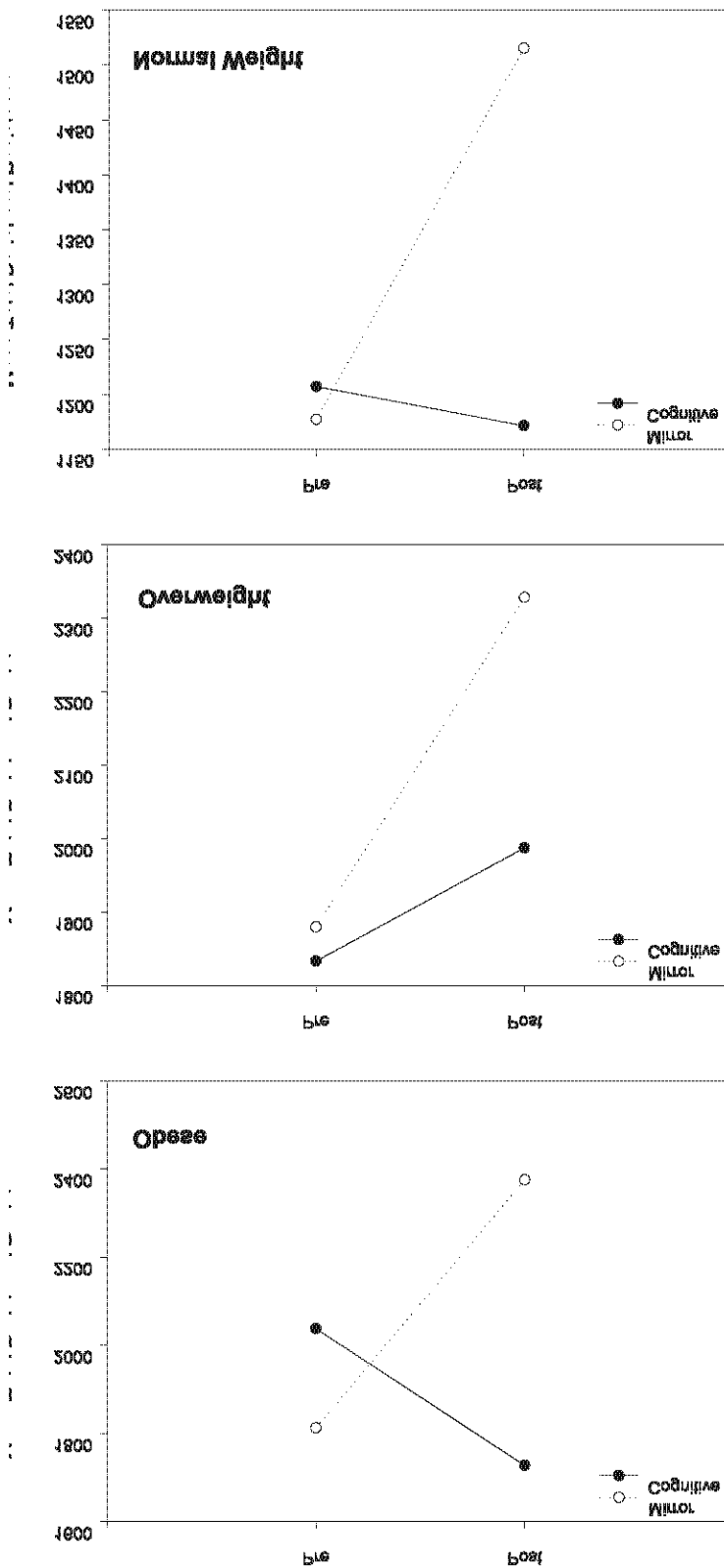
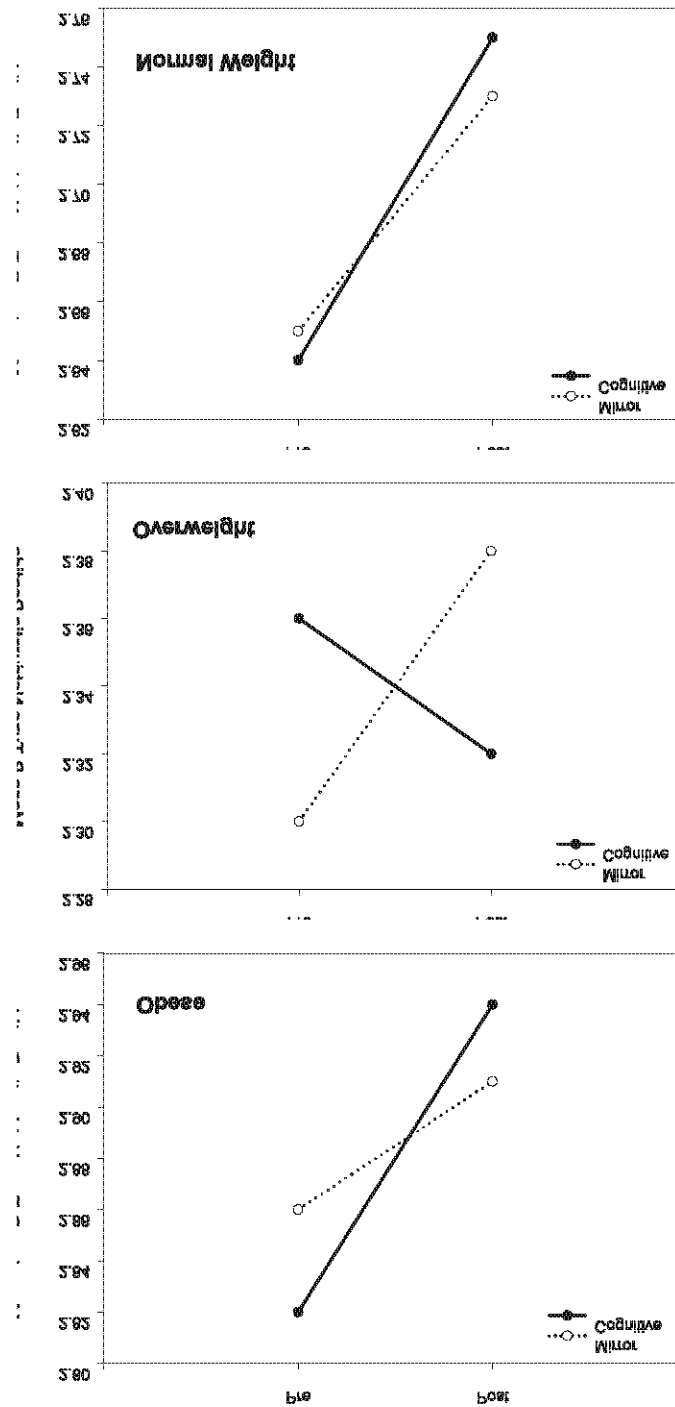
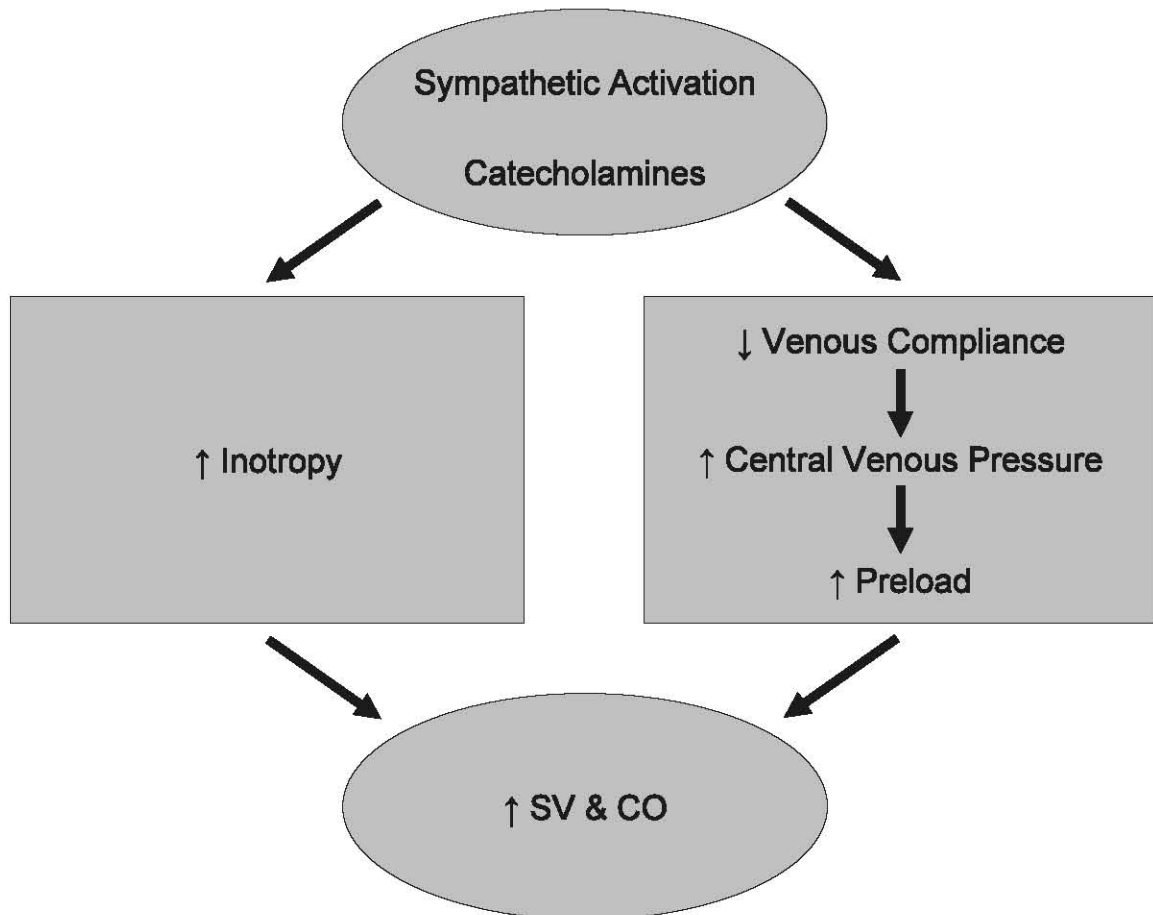


Figure 15. Log-transformed BNP (pg/mL) Response to Both Mental Stressors Presented Separately for the Three Weight Groups



*Figure 16.* Physiologic Pathways Proposed as a Mechanism by Which Sympathetic Activation During Cognitive/Emotional Stress Increases Stroke Volume (SV) and Cardiac Output (CO)



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